Journal of Ethnopharmacology 119 (2008) 376–412

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Review

South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides*—A review

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ARTICLE INFO

Article history:
Received 7 May 2008
Received in revised form 10 June 2008
Accepted 13 June 2008
Available online 22 June 2008

Keywords:
Anticarcinogenic
Antioxidant
Bush tea
Chemical composition
Honeybush
Phyto-oestrogenic
Polyphenols
Rooibos

ABSTRACT

Rooibos (*Aspalathus linearis* (Brum.f) Dahlg.) and honeybush (*Cyclopia* Vent. species) are popular indigenous South African herbal teas enjoyed for their taste and aroma. Traditional medicinal uses of rooibos in South Africa include alleviation of infantile colic, allergies, asthma and dermatological problems, while a decoction of honeybush was used as a restorative and as an expectorant in chronic catarrh and pulmonary tuberculosis. Traditional medicinal uses of *Athrixia phylicoides* DC., or bush tea, another indigenous South African plant with very limited localised use as herbal tea, include treatment of boils, acne, infected wounds and infected throats. Currently rooibos and honeybush are produced for the herbal tea market, while bush tea has potential for commercialisation. A summary of the historical and modern uses, botany, distribution, industry and chemical composition of these herbal teas is presented. A comprehensive discussion of *in vitro*, *ex vivo* and *in vivo* biological properties, required to expand their applications as nutraceutical and cosmeceutical products, is included, with the main emphasis on rooibos. Future research needs include more comprehensive chemical characterisation of extracts, identification of marker compounds for extract standardisation and quality control, bioavailability and identification of bio-markers of dietary exposure, investigation of possible herb–drug interactions and plant improvement with regards to composition and bioactivity.

Contents

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doi:10.1016/j.jep.2008.06.014

1. Introduction

A number of plants have traditionally been used as a "tea" in South Africa (Watt and Breyer-Brandwijk, 1932), but only the two Cape fynbos plants, *Aspalathus linearis* (Brum.f) Dahlg. (family Fabaceae; tribe Crotalarieae), better known as rooibos, and various species of *Cyclopia* Vent. (family Fabaceae; tribe Podalyrieae), commonly known as honeybush, have enjoyed commercial success as herbal teas. Recently, interest was shown in *Athrixia phylicoides* DC. (family Asteraceae, tribe Gnaphalieae), another indigenous South African plant with potential for commercialisation (Rampedi and Olivier, 2005). It is most commonly known as bush tea or Zulu tea. Table 1 summarises the common names of the different plants used as teas.

These species represent the full spectrum of the South African herbal tea industry, from an established (rooibos) and developing (honeybush) industry to a product (bush tea) mainly gathered for home use in rural areas and sold by informal traders in urban areas. In the case of rooibos and honeybush their use evolved from medicinal to nonmedicinal, i.e. herbal tea drunk for enjoyment, to the present day situation where "food as medicine" has brought the focus back to medicinal properties, albeit now driven from the perspective of Western society and present day health problems.

On the other hand, the use of bush tea, both for medicinal and nonmedicinal purposes, is limited to native peoples of South Africa. Urbanisation has spread the demand for bush tea to urban areas such as Gauteng, where traditional healers still prescribe *Athrixia*-based medicines for a number of ailments. It has not yet made the transition to the mainstream herbal tea market, but the "success stories" of rooibos and honeybush encouraged investigation into commercialisation (Jana Olivier, UNISA, Pretoria, South Africa, 2005; personal communication).

This review will give an overview of the historical and modern uses, botany, distribution, industry, including quality control, and the chemical composition of these three South African herbal teas. Furthermore, the review attempts to discuss all literature to date pertaining to the biological properties of rooibos (Table 2), honeybush (Table 3) and bush tea that have implications for health.

2. Historical and modern uses

Watt and Breyer-Brandwijk (1932) listed rooibos as a South African medicinal plant, but no indication of specific application is given. The botanist Carl Thunberg reported that, during his travels in Africa in 1772, he met the Khoi who used it as a beverage (Morton, 1983). This practice was also observed by Benjamin Ginsberg in the early 1900s when he met descendants of the Khoi in the Clanwilliam region of the Western Cape who, during the summer months, harvested the plants growing wild in the mountains. They chopped the shoots using an axe, crushed them with a mallet and "sweated" ("fermented") the bruised pieces in the hollows of stone reefs, followed by sun-drying. This process formed the basis of the manufacturing process for fermented rooibos that is still practiced today.

The modern use of rooibos as an herbal tea, mostly enjoyed as an alternative to Oriental tea, originated at the beginning of the previous century. It was consumed as a strong, hot brew with milk and sugar added. Preparation entailed boiling of the leaves and stems in water whereafter the brew was kept hot at low heat. After each serving, more water or leaves and stems, depending on taste, was added to the pot. The modern day consumers, with convenience in mind, mostly use tea bags instead of loose-leaf tea and prepares the infusion in the same manner as Oriental tea, i.e. infusing one tea bag (ca. 2 g) per cup with freshly boiled water for 2–5 min to release flavour and colour, which is then served hot, with or without milk and sugar added according to taste. Japanese consumers drink it very diluted compared to South African consumers. Their normal preparation procedure entails the boiling of 3.5–4.5 g per 2 l of water for 20 min (Ito et al., 1991; Komatsu et al., 1994).

In South Africa during summer it is also enjoyed cold, usually with lemon juice and sugar added. Rooibos chai and mixtures with other herbs (e.g. honeybush, buchu, fennel or *Sutherlandia frutescens*) are some of the tea bag products on the market. Vanilla

Table 1
Botanical classification and common names of *Aspalathus linearis*, *Cyclopia* species and *Athrixia* species

Family	Species	Common names	Reference
Fabaceae	*Aspalathus linearis* (Brum.f) R. Dahlgr. (syns. *Aspalathus contaminatus* (Thb.) Druce; *Bordonia pinifolia* Marl.; *Psoralea linearis* Burm.)	Red tea; Koopmanstee, naaldtee, rooibostee, speldtee, swarttee [Afrikaans]	Watt and Breyer-Brandwijk (1932), Kies (1951) and Dahlgren (1968)
	Cyclopia longifolia Vog.	Bush tea, honey tea, boer tea; bostee, bossiestee, heuningtee [Afrikaans]	Watt and Breyer-Brandwijk (1932)
	Cyclopia latifolia DC.	Bush tea, honey tea, boer tea; bostee, bossiestee, heuningtee [Afrikaans]	Watt and Breyer-Brandwijk (1932)
	Cyclopia genistoides (L.) Vent.	Bush tea, honey tea, boer tea; bostee, bossiestee, heuningtee; Overbergtee[a], kustee[a] [Afrikaans]	Watt and Breyer-Brandwijk (1932)
	Cyclopia bowieana Harv.	Vleitee [Afrikaans]	Kies (1951)
	Cyclopia maculata (Andr.) Comb. Nov.	Vleitee [Afrikaans]	
	Cyclopia sessiliflora Eckl. and Zeyh.	Gewone bossiestee, Heidelbergtee[a] [Afrikaans]	Kies (1951)
	Cyclopia subternata Vog.	Gewone bossiestee, vleitee[a] [Afrikaans]	Kies (1951)
	Cyclopia intermedia E. Mey.	Kouga bush tea; bergtee[a] [Afrikaans]	Kies (1951)
Asteraceae	*Athrixia phylicoides* DC.	Bush tea, Bushman's tea, Zulu tea; itshalo, umtshanela, iphephetha, ishanela, ishayelo [Zulu]; iCholocholo [Xhosa, Zulu]; sephomolo [Sotho, Swazi], luphephetse [Swazi]	Watt and Breyer-Brandwijk (1962), Fox and Young (1982), Van Wyk and Gericke (2000) and Anon. (2008)
	Athrixia elata Sond.	Daisy tea; wildetee, bostee [Afrikaans], phefshoana-ea-basiea [Sotho]	Watt and Breyer-Brandwijk (1962), Fox and Young (1982), Van Wyk and Gericke (2000) and Anon. (2008)

[a] Common names that are presently in use (ARC, 2008). *Cyclopia sessiliflora* was also described as Heidelbergtee by Kies, 1951; tee [Afrikaans] = tea.

flavoured rooibos in tea bags is more readily available in Europe than unflavoured rooibos. Dos et al. (2005) showed that Turkish consumers had no significant preference for vanilla-flavoured rooibos over the unflavoured, natural product, or for a 5 min brew over a 5 min infusion. Several ready-to-drink rooibos iced teas have entered the market during the past few years. The latest concept, borrowed from coffee and gaining popularity in coffee and tea shops, is rooibos served as "espresso, cappuccino, or latte" (Anon., 2006).

Rooibos tea was considered healthy, based on the fact that it was "harmless and satisfying" due to the absence of alkaloids and a low tannin content (Cheney and Scholtz, 1963). It was only after the discovery by Annetjie Theron in 1968 that an infusion of rooibos, when administered to her colicky baby, cured the chronic restlessness, vomiting and stomach cramps, that rooibos became well-known as a "healthy" beverage, leading to a broader consumer base. Many babies since have been reared with rooibos either added to their milk, or given as a weak brew. Anecdotal evidence suggests that it has an anti-allergic effect, improves the appetite, reduces nervous tension and promotes sound sleep (Morton, 1983). It also alleviates ingestion, heartburn and nausea (Van Wyk et al., 1997). Topical applications of rooibos extract are believed to alleviate dermatological problems, i.e. eczema, acne and nappy rash, leading to the development of toiletries and cosmetic products, which are sold through supermarkets, farms stalls and beauty shops. Annetjie Theron was the first to develop a range of skin-care products containing rooibos extract that eventually became well-established in South Africa.

During his travels Thunberg also came across "Honigtee". *Cyclopia genistoides* was the earliest of the *Cyclopia* species to be used as a tea at the Cape (Smith et al., 1966). Greenish (1881) noted that *Cyclopia genistoides* (Cape tea, "honig-thee") was used as a substitute for tea. Although *Cyclopia vogelii* was principally used for tea preparation (Marloth, 1913), Kies (1951) noted *Cyclopia genistoides* as the most common tea plant. *Cyclopia vogelii* was later renamed to *Cyclopia subternata* (Watt and Breyer-Brandwijk, 1962). Other species with a history of use as tea by the colonists were *Cyclopia latifolia* and *Cyclopia longifolia* (Marloth, 1913, 1925). In the 1920s all the tea made on the Cape Peninsula was from *Cyclopia genistoides*, while in the Caledon (Overberg) and George areas *Cyclopia subternata* was used (Marloth, 1925).

Watt and Breyer-Brandwijk (1932) noted medicinal properties for *Cyclopia genistoides* only. A decoction of the plant was used as a restorative and as an expectorant in chronic catarrh and pulmonary tuberculosis (Bowie, 1830). Drinking an infusion of honeybush apparently also increases the appetite, but no indication is given of the specific species (Kobert, 1906 as cited by Watt and Breyer-Brandwijk, 1932). According to Marloth (1925) honeybush was praised by many colonists as being wholesome, valuing it as a stomachic that aids weak digestion without producing any serious stimulating effects of the heart. It also alleviates heartburn and nausea (Van Wyk et al., 1997). Anecdotal evidence suggests that it stimulates milk production in breast-feeding women and treats colic in babies (Rood, 1994).

Modern use of honeybush followed the example of rooibos, and in many instances it is enjoyed as an infusion prepared from a mixture of honeybush and rooibos. The tea bags usually contain a larger percentage of rooibos than honeybush. Parts of other indigenous South African plants and fruits mixed with honeybush include dried buchu leaves, pieces of African potato (*Hypoxis hemerocallidae*) corms and dried marula (*Sclerocarya birrea*) fruit. The ready-to-drink honeybush iced tea market is not developed to the same extent as that of rooibos, while honeybush "espresso", etc. has not been tried. The number of available toiletries and cosmetic products lags, at this stage, behind rooibos.

The dried leaves and fine twigs of *Athrixia phylicoides* have traditionally been used by the Khoi and Zulu people as an herbal tea and medicinal decoction (Van Wyk and Gericke, 2000). Watt and Breyer-Brandwijk (1962) reported that the Zulu and people of European ancestry took the infusion as a "blood purifier" for sores, boils and the like. The decoction has been used as a washing agent or lotion for treating sore feet, boils, acne and infected wounds, as well as a stimulant and an aphrodisiac drink (Hutchings et al., 1996; Van Wyk and Gericke, 2000). It was used as a gargle for infected throats and a decoction of the roots served as a purgative and cough

Table 2
Chronological record of the major findings of investigations dealing with the potential health benefits of *Aspalathus linearis* extracts as demonstrated *in vitro* using subcellular fractions and cell culture, and *in vivo* using animal models

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Fermented	*In vivo* (human)	2.5 g leaves/100 ml freshly boiled water, steeped for 5 min	No effect on iron absorption using ^{59}Fe isotope absorption followed by single serving of 200 ml with 40 ml milk and 20 sugar added to 30 males between 21 and 34 years of age	Hesseling et al. (1979)
Fermented	*In vivo*	Water extract 220–230 stonger than a normal cup of rooibos	Highly concentrated water extract mutagenic using *Drosphila melanogaster* Extract at normal "tea concentration" not mutagenic	Neethling et al. (1988)
Fermented	*In vitro*	1.13 g leaves/100 ml water, boiled for 20 min	Pre-treatment of mouse L5178Y cells with extract for 2 h before exposure to H_2O_2 protected against inactivation; protective effect dose-dependant with highest protection by undiluted extract No protective effect when cells were treated simultaneously with extract and H_2O_2	Ito et al. (1991)
Fermented	*In vivo* (human)	0.2 g leaves/100 ml water, boiled for 20 min	Weekly consumption of 1500 ml tea shown to be beneficial to patients with dermatological diseases: Decreased the incidence of recurrent *herpes simplex* Decreased incidence of incurable *verruca plana juvenilis* and *verruca vulgaris* (human papilloma virus infection) Decreased itching caused by *prurigo nodularis* Inhibited infiltration of neutrophils associated with Behcet's disease, *psoriasis vulgaris* and *acne pustulosa*	Shindo and Kato (1991)
Fermented	*In vitro*	3.33 g leaves/100 ml water, boiled for 15 min	Suppressed chromosome aberrations in Chinese hamster ovary cells induced by MMC or B(a)P in the presence or absence of rat liver microsomal enzymes (S9). The clastogen-suppressing effects was obtained when cells were exposed to rooibos before and/or after mutagen treatment	Sasaki et al. (1993) and Shimoi et al. (1994)
Fermented	*In vivo*	3.33 g leaves/100 ml water, boiled for 15 min and freeze-dried	Reduced formation of micronucluated reticulocytes (MNRETs) in peripheral blood of ICR male mice by mitomycin C (MMC) after single i.p (0.1% extract, 1 ml/mouse) administration 6 h prior to mutagen treatment. Oral administration [0.05% extract, 1 ml/mouse)] for 28 days to ICRmale mice decreased the frequency of B(a)P and MMC-induced MNRETs	Sasaki et al. (1993) and Shimoi et al. (1994)
Fermented	*In vitro*	3.33 g leaves/100 ml water, boiled for 15 min and freeze-dried	Freeze-dried extract (10–50 mg/50 ml PBS) suppressed formation of glycated albumin, including AGEs, in a mixture of D-glucose (5 g/50 ml) and human serum albumin (500 mg/50 ml)	Kinae et al. (1994)
Fermented	*In vitro*	0.175 g leaves/100 ml water, boiled for 20 min (=100% extract)	Suppressed oncogenic transformation of C3H10T1/2 mouse embryo fibroblast cells induced by X-rays dose dependently (2–10% of the extract); cells were treated with extract directly after irradiation	Komatsu et al. (1994)
Fermented	*In vivo*	3.33 g leaves/100 ml water, boiled for 15 min and freeze-dried	Oral administration for 28 days (0.05% extract *ad libitum)* reduced frequency of γ-ray irradiation (1.5 Gy)-induced MNRETs in mouse	Shimoi et al. (1994)
Fermented	*In vivo*	Not specified	Suppressed age-related accumulation of lipid peroxides in the brain of aged female Wistar rats receiving tea daily *ad libitum* (tea concentration not specified) from the age of 3 months until 24 months old	Inanami et al. (1995)
Fermented	*In vivo*	3.33 g leaves/100 ml water, boiled for 20 min; column fractionation	Single gastric intubation (1 ml extract or flavonoid fraction) 2 h prior to γ-ray irradiation reduced frequency of MNRETs in ICR mice	Shimoi et al. (1996)
Fermented	*In vitro*	0.175 g leaves/100 ml water, boiled for 20 min (=100% extract)	Dose-dependant inhibition of cell proliferation of primary embryonic skeletal muscle cells, fibroblasts and myoblasts by 2, 10 and 100% (undiluted) extract Ornithine decarboxylase activity affected only by 100% extract	Lamošová et al. (1997)
Fermented	*In vitro*	Water extract—1 g leaves/100 ml, extracted for 3 h at 85 °C; Alkaline extract—10 g water-extracted leaves/100 ml 1% $NaCO_3$ extracted for 3 h at 45 °C	Alkaline extract (EC_{50} = 38.9 μg/ml) suppressed HIV-induced cytopathicity of HIV (HTLV-III) infected MT-4 cells, but hot water extract ineffective	Nakano et al. (1997a,b)

Table 2 (*Continued*)

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Fermented	*In vitro*	Quantities not available; extract was freeze-dried	Dose-dependent inhibition of lipid peroxidation in cell membranes using rabbit erythrocyte membrane (85% inhibition at 1 mg/ml), rat liver microsome (87% inhibition at 0.2 mg/ml) and rat liver homogenate	Hitomi et al. (1999)
Unfermented; fermented	*In vitro*	2 g leaves/100 ml freshly boiled water, steeped for 30 min and freeze-dried	Extract (5 and 10%) inhibited 2-AAF- and AFB_1-induced mutagenesis in the *Salmonella typhimurium* mutagenicity assay (tester strains TA98 and TA100) in the presence of metabolic activation with S9 Unfermented extract more effective than fermented extract Poor inhibitory effect against direct acting mutagens, MMS, cumolhydroperoxide and H_2O_2, using tester strain TA102 Unfermented extract displayed a comutagenic effect against MMS	Marnewick et al. (2000)
Fermented	*In vitro*	RT_A—0.175 g leaves/100 ml freshly boiled water, steeped for 30 min RT_B—0.175 g leaves/100 ml water boiled for 10 min, followed by steeping for 20 min	RT_A extract dose-dependently inhibited peroxide-induced hemolysis of Japanese quail erythrocytes similar to ascorbic acid RT_B extract marginally less protective than RT_A extract (both at 175 mg/100 ml); protective effect not significant different from ascorbic acid (14 mg/100 ml)	Simon et al. (2000)
Fermented	*Ex vivo*	Milled plant material; 0.175 g leaves/100 ml freshly boiled water, steeped for 30 min	Supplementation of diet with milled plant material (3.1 g/kg) or extract supplied as sole drinking fluid for 6 months did not affect the fragility of erythrocyte membranes of Japanese quails treated with peroxide and hypotonic NaCl solution	Simon et al. (2000)
Fermented	*In vitro*	1.63 g leaves/100 ml water, boiled for 15 min and freeze-dried	Primary OVA and SRBC antibody responses in murine splenocytes markedly increased or decreased, depending on dose (1–100 μg/ml) No effect on LPS-elicited antibody production by splenic B cells Dose-dependently (10–1000 μg/ml) increased IL-2 secretion by OVA-primed murine splenocytes Dose-dependently increased anti-CD3ε Ab-induced IL-2 secretion by OVA-primed murine splenocytes Inhibited IL-4 secretion by OVA-primed murine splenocytes	Kunishiro et al. (2001)
Fermented	*Ex vivo*	0.163 g leaves/100 ml water, boiled for 15 min	OVA-induced IL-2 secretion increased in splenocytes of female BALB/c mice treated with rooibos (4 ml/day) extract for 3 weeks	Kunishiro et al. (2001)
Fermented	*In vivo*	0.163 g leaves/100 ml water, boiled for 15 min	Extract administration as sole drinking fluid (*ad libitum*, consumption 30 ml/day) for 1 week prior and 2 weeks after largely restored the reduction of cyclosporin A-induced OVA antibody production (IgM) in the serum of female Wistar/ST rats	Kunishiro et al. (2001)
Different processing stages	*In vitro*	10 g leaves/100 g water, boiled for 30 min and freeze-dried	Extract (0.02–0.1 mg/ml) inhibited 2-AAF-induced mutagenesis in the *Salmonella typhimurium* assay (tester strain TA98) in the presence of metabolic activation with S9 Fermentation reduced antimutagenicity of extracts	Standley et al. (2001)
Fermented	*In vitro*	5 g leaves/100 g water, boiled for 10 min	Extract reduced genotoxicity of 2-AAF and PhIP in V79 cells of Chinese hamsters expressing rat CYP1A2 and SULT1C1 Extract moderately inhibited the genotoxicity of BaP-7,8 OH and N–OH–PhIP (Comet assay)	Edenharder et al. (2002)
Unfermented; fermented	*In vivo*	2 g leaves/100 ml freshly boiled water, steeped for 30 min	Extract administered as sole drinking fluid (*ad libitum*) to male Fischer 344 rats for 10 weeks (controls received water): Unfermented and fermented extract enhanced cytosolic glutathione *S*-transferase α in rat liver Unfermented extract significantly enhanced microsomal UDP-GT activity in rat liver Unfermented and fermented extracts decreased the levels of GSSG in rat liver significantly, while GSH was markedly increased resulting in an increase in the GSH/GSSG ratio Did not alter liver function enzymes AST, ALT and ALP Did not alter levels of total bilirubin, unconjugated bilirubin, and total protein Did not alter levels of serum creatinine, total cholesterol and total plasma iron	Marnewick et al. (2003)

Table 2 (*Continued*)

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Fermented	*In vivo*	0.25 g leaves/100 ml water, boiled for 10 min and steeped for 20 min	Extract administered as sole drinking fluid (*ad libitum*) (consumption ca 32 ml/day) to male Wistar rats for 7 days before treatment with CCl_4 protected against hepatic injury: Histological regression of steatosis and cirrhosis in the liver tissue Suppressed increase in plasma activities of aminotransferases (ALT, AST) and alkaline phosphate and billirubin concentrations Reduced increase in plasma MDA, triacylglycerols and cholesterol	Uličná et al. (2003)
Fermented	*In vivo*	Freeze-dried hot water extract	Dietary supplementation of vitamin E deficient [VE(−)] male Wistar rats with extract (3 g/100 g) for 8 weeks: Decreased (ns) lipid peroxidation in liver, small interstine, lung, testis and muscle Tissue showed lower susceptibility for AAPH-induced lipid peroxidation Kidney glutathione peroxidase decreased (ns) by extract as compared to VE(−) control	Hitomi et al. (2004)
Supplement	*In vivo*	Not available	Supplement (2 g/kg diet) fed for 3 days, suppressed liver CYP2C11 activity in male Sprague–Dawley rats	Jang et al. (2004)
Fermented	*In vivo*	0.25 g leaves/100 ml water, boiled for 10 min	Extract administered (*ad libitum*) as drinking fluid to male Wistar rats and by gavage (5 ml/kg body weight once a day) for 7 days before CCl_4 treatment: Restored liver concentrations of CoQ_9H_2 and α-tocopherol to levels comparable to healthy animals Inhibited formation of MDA in liver Did not counter the decrease in liver CoQ_9	Kucharská et al. (2004)
Fermented	*In vitro*	Water extract—5 g leaves/100 ml water, refluxed at 80 °C and freeze-dried; 75% ethanol extract, freeze-dried	Both extracts dose-dependently protected pBR 322 plasmid DNA against strand scission caused by peroxyl radical-induced oxidative stress; water and 75% ethanol extracts (5 μg/ml) prevented DNA strand scission by 70 and 87%, respectively	Lee and Jang (2004)
Unfermented; fermented	*Ex vivo*	2 g leaves/100 ml freshly boiled water, steeped for 30 min	Modulation of chemical-induced mutagenesis in the *Salmonella* mutagenicity assay by subcellular fractions of male Fischer rats treated with extract for 10 weeks: Protected cytosolic and microsomal liver fractions of rats against AFB_1-induced mutagenesis Microsomal fraction does protected against AFB_1-induced but not against 2-AAF-induced mutagenesis	Marnewick et al. (2004)
Unfermented; fermented	*In vivo*	2 g leaves/100 ml freshly boiled water, steeped for 30 min	Extract administered (*ad libitum)* to male Fischer 344 rats 1 week after cancer initiated by diethylnitrosamine, followed by fumonisin B_1-cancer promoting diet (250 mg fumonisin B_1/kg feed) for 3 weeks inhibited the number and size of DEN-initiated pre-neoplastic liver lesions Fermentation of plant material reduced protective effect of extract Unfermented rooibos reduced the induction of GSH, GSSG, GSH:GSSG ratio and ORAC Fermented rooibos reduced lipid peroxidation in the liver	Marnewick (2004)
Fermented	*In vitro*	Methanol extract	Extract (1, 5, 10 μg/ml) inhibited TPA-induced COX-2 expression in human epithelial (MCF10A) cells Inhibited TPA-induced COX-2 expression through suppression of DNA binding of NF-κB	Na et al. (2004)
Fermented	*In vivo*	Methanol extract	Extract (60, 300 and 600 μg), topically applied prior to TPA, inhibited TPA-induced COX-2 expression in the skin of female ICR mice	Na et al. (2004)
Unfermented	*In vivo* (human)	80% ethanol extract, decolourized and containing 16% aspalathin	A twice daily dose of extract (250 mg/tablet) in addition to a restricted flavonoid diet followed by 20 healthy male and female smokers and non-smokers for 2 weeks: No effect on blood parameters No effect on antioxidant status of plasma measured using ABTS and Cu^{2+}-induced LDL oxidation assays Decreased plasma antioxidant status measured using xanthine/xanthine oxidase assay	Sauter (2004)

Table 2 (*Continued*)

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Fermented	*In vivo* (human)	50 tea bags/20 liter freshly boiled water	Intake of tea (2× 200 ml/day with milk and sugar added) by 175 children over a 16-week period did not affect the iron status, i.e. serum ferritin, transferrin, total iron binding capacity and transferrin saturation	Breet et al. (2005)
Unfermented; fermented	*In vivo*	Defatted plant material exhaustively extracted with methanol; evaporated; dried methanol residue reconstituted in 1:1 ethanol and acetone mixture (30 mg/1 ml)	Ethanol/acetone soluble fraction of a methanol extract (2.24–2.29 mg/mouse) prior to tumour promotor with TPA after initiation with DMBA inhibited tumour (papilloma) development Fermented extract more protective in terms of reducing the number and size of papillomas	Marnewick et al. (2005)
Fermented	*In vitro*	1.5 g leaves/100 ml water, boiled for 10 min, steeped for 20 min; evaporated; reconstituted in water	Extract concentration-dependently (1–10 mg/ml) relaxed spontaneous and K^+-induced contractions of rabbit jejunum Posessed K_{ATP} channel and weak Ca^{2+} antagonist activity presumably due to the flavonoids, chrysoeriol, orientin and vitexin	Gilani et al. (2006)
Fermented	*In vivo*	1.5 g leaves/100 ml water, boiled for 10 min, steeped for 20 min; evaporated; reconstituted in saline	Extract dose-dependently (500 and 1000 mg/kg) (i.p.) reduced antidiarrhoeal antisecretory activities in caster oil (10 mg/kg)-induced diarrhoea model in male and female Balb-C mice	Gilani et al. (2006)
Fermented	*In vitro*	1.5 g leaves/100 ml water, boiled for 10 min, steeped for 20 min; evaporated; reconstituted in water	Extract exhibited bronchodilator and antispasmodic effects via K_{ATP} channel activation in rabbit jejunum and aorta, guinea-pig atria and trachea Chrysoeriol indicated as bioactive compound	Khan and Gilani (2006)
Fermented	*In vivo*	1.5 g leaves/100 ml water, boiled for 10 min, steeped for 20 min; evaporated; reconstituted in water	Extract dose-dependently (10, 30, 100 mg aqueous extract/kg, i.v.) reduced mean arterial blood pressure of male and female Sprague–Dawley rats	Khan and Gilani (2006)
Fermented	*In vitro*	5 g/100 ml freshly boiled phosphate-buffered saline, steeped for 10 min	Effect on ACE activity and NO production in cultured human umbilical veins endothelial cells: No inhibition of ACE after 10 min incubation in the presence of rooibos extract Dose dependently increased NO production after 24 h incubation	Persson et al., 2006
Unfermented	*In vitro*	Leaves extracted with methanol; preparation of 80% methanol extract by partitioning; 50% and 100% methanol column fractions	Oestrogenic activity demonstrated for 80% methanol layer and 50 and 100% methanol eluates with oestrogen ELISA assay	Shimamura et al. (2006)
Fermented	*In vivo*	Water extract—0.25 g leaves/100 ml water, boiled for 10 min and steeped for 20 min Alkaline extract—10 g water-extracted leaves/100 ml 1% $NaCO_3$ extracted for 3 h at 45 °C	Water extract fed *ad libitum* plus additional once daily gavage of 5 ml/kg body weight did not alter diabetic status induced by streptozotocin in male Wistar rats Alkaline extract (300 mg/kg body weight; gavaged) did not alter diabetic status induced by streptozotocin in male Wistar rats Both extracts significantly decreased plasma creatinine Both extracts lowered advanced glycation end-products (AGEs) and lipid peroxidation (MDA) in the plasma and lens; water extract also decerased MDA in the liver	Uličná et al. (2006)
Unfermented; fermented	*In vitro*	10 g leaves/100 ml freshly boiled water, steeped for 5 min; freeze-dried	Extract (0.5–5 mg/plate) inhibited 2-AAF- and AFB_1-induced mutagenesis in the *Salmonella typhimurium* assay (tester strain TA98 and TA100, respectively) in the presence of metabolic activation with S9 Fermentation enhanced antimutagenicity (see also Standley et al., 2001)—discrepancy between results of two studies emphasises role of phenolic variation in plant material on the measured effect	Van der Merwe et al. (2006)
Fermented	*In vitro*	1.75 g leaves/100 ml water, boiled for 15 min, freeze-dried. Column fractionation (100% water eluate = fraction A)	Hydrophilic fraction (A) increased IgM production in anti-OVA-stimulated murine splenocytes	Ichiyama et al. (2007)
Fermented	*In vivo*	1.75 g leaves/100 ml water, boiled for 15 min, freeze-dried. Column fractionation (100% water eluate = fraction A)	Hydrophyllic fraction (A) (160 μg/ml) ingested at a dose of 3–5 ml/day increased anti-OVA IgM level in sera of BALB/c mice: Effects associated with presence of oligo- and polysaccharides	Ichiyama et al. (2007)

Table 2 (*Continued*)

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Fermented	*In vivo*	0.4 g leaves/100 ml water, simmered for 5 min	Extract administered *ad libitum* to male Sprague–Dawley rats for 2 weeks prior to oral administration of midazolam (a CYP3A probe substrate): Increased the intestinal 1′- and 4-hydroxylation activities of CYP3A Increased intestinal CYP3A content Slightly increased hepatic midazolam hydroxylation and CYP3A content	Matsuda et al. (2007)
Fermented	*Ex vivo*	0.4 g leaves/100 ml water, simmered for 5 min	Herb–drug interactions demonstrated using intestinal and hepatic microsomes from male Sprague–Dawley rats after feeding extract as sole source in drinking water for 2 weeks: CYP3A content (western blot) significantly increased in the intestine. 50% increased intestinal hydroxylation of midazolam by CYP3A Slight increased hepatic hydroxylation of midazolam and CYP3A content	Matsuda et al. (2007)
Unfermented; fermented	*In vitro*	10 g leaves/100 ml freshly boiled water, steeped for 5 min; freeze-dried	Extract (0.02 mg/ml) protected rat liver microsomes against Fe^{2+}-induced lipid peroxidation	Joubert et al. (2008b)
Fermented	*In vivo*	0.175 g leaves/100 ml water, boiled for 10 min, followed by steeping for 20 min	Extract fed *ad libitum* or supplementation of feed with milled plant material (3.5 g/kg) prolonged productive life of aged Japanese quails (model for ageing studies)	Juráni et al. (2008)
Unfermented; fermented	*In vitro*	Commercial aqueous extracts fractionated by hydrophobic interaction chromatography	Fermented extracts inhibited effects of mixed type progesterone binding to CYP21 Hydrophobic extracts showed stronger competitive inhibition than more hydrophilic fractions. Fermented rooibos exhibited more potent inhibitory effects while phenolic components showed no effect RP-HPLC fractions showed strong non-competitive binding. More hydrophobic fractions showed stronger inhibition	Richfield (2008)
Unfermented; fermented	*In vivo*	2 g leaves/100 ml freshly boiled water, steeped for 30 min; freeze-dried	Extract protected against methylbenzylnitroasamine-induced male Fischer 344 rat oesophageal papillomas by reducing number and size Protective effects reduced by fermentation	Sissing (2008)
Unfermented	*In vitro*	2 g leaves/100 ml freshly boiled water, steeped for 30 min; freeze-dried	Extract of unfermented rooibos inhibited cell proliferation of human oesophageal cancer cells (WHCO5) by affecting the energy (ATP) production Cytotoxicity (IC_{50}) reduced by fermentation	Sissing (2008)
Fermented	*In vitro*	10 g leaves/100 ml 70% aqueous methanol, sonicated for 30 min	Extract (50, 100 and 200 μg/ml) increased viability of Chinese hamster fibroblast (V79-4) cells following H_2O_2-induced oxidative stress in a non-dose response manner Extract (100 μg/ml) protected against inhibition of GJIC in V79-4 cells following H_2O_2-induced oxidative stress Extract (100 μg/ml) increased activity of SOD and CAT inV79-4 cells	Yoo et al. (2008)

Four human studies are included.

Abbreviations: 2-AAF: 2-acetylaminofluorene; ABTS: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid; ACE: angiotensin-converting enzyme; AFB_1: aflatoxin B_1; AGEs: advanced glycation end products; B(*a*)P: benzo[*a*]pyrene; CAT: catalase; DMBA: 7,12-dimethylbenz(*a*)anthracene; GJIC: gap-junction intercellular communication; IgM: immunoglobin M; IL: interleukin; LDL: low-density lipoprotein; LPS: lipopolysaccharide-stimulated; MDA: malondialdehyde; MMC: mitomycin C; MMS: methyl methanesulphonate; MNRETs: micronucleated reticulytes; NO: nitric oxide; ns: not significantly; OVA: ovalbumin; PhIP: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SOD: superoxide dismutase; SRBC: sheep red blood cell; TPA: 12-*O*-tetradecanoylphorbol-13-acetate.

[a] Leaves indicate plant material as sold commercially which include varying quantities of stems.

remedy. Leaves were also chewed and the juice swallowed as a cough remedy (Watt and Breyer-Brandwijk, 1962). According to two recent surveys its medicinal use by black people in rural and urban areas is now limited (Rampedi and Olivier, 2005). Rural respondents of the surveys use it to treat hypertension, heart disease, diabetes, diarrhoea, vomiting and skin complaints. It also acts as a "blood purifier" and an anthelmintic. Urban respondents claimed treatment of circulation and heart problems, as well as "cleansing" and revitalising properties. Skin conditions such as abscesses, boils and sores are treated by external application. According to the same surveys, a larger proportion of the rural and urban respondents use it as a tea rather than for medicinal purposes, with some drinking it every day. It is most frequently consumed by older women. Of the different ethnic groups questioned in Gauteng, the North-

Table 3
Chronological record of the major findings of investigations dealing with the potential health benefits of *Cyclopia* extracts as demonstrated *in vitro* using subcellular fractions and cell culture, and *in vivo* using animal models

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Unfermented; fermented	*In vitro*	4 g leaves/100 ml freshly boiled water, steeped for 30 min and freeze-dried	*Cyclopia intermedia* extract (5 and 10%) inhibited 2-AAF- and AFB_1-induced mutagenesis in *Salmonella typhimurium* mutagenicity assay (tester strains TA98 and TA100, respectively) in presence of metabolic activation (S9) Fermentation reduced inhibitory effect *Cyclopia intermedia* displayed weak inhibitory effect against direct acting mutagens, MMS, cumolhydroperoxide and H_2O_2, using tester strain TA102	Marnewick et al. (2000)
Unfermented; fermented	*In vivo*	4 g leaves/100 ml freshly boiled water, steeped for 30 min	*Cyclopia intermedia* extract fed as sole drinking fluid to male Fischer 344 rats for 10 weeks (controls received water): Unfermented and fermented extracts enhanced cytosolic glutathione *S*-transferase α in rat liver Unfermented extract enhanced microsomal UDP-GT activity in rat liver Unfermented and fermented extracts decreased GSSG levels in rat liver and markedly increased GSH levels resulting in increased GSH/GSSG ratio Did not alter activity of liver function enzymes AST, ALT and ALP Did not alter serum levels of total bilirubin, unconjugated bilirubin, and total protein Did not alter serum levels of creatinine, total cholesterol and total plasma iron	Marnewick et al. (2003)
Unfermented; fermented	*Ex vivo*	4 g leaves/100 ml freshly boiled water, steeped for 30 min	Modulation of chemical-induced mutagenesis in the *Salmonella* mutagenicity assay by subcellular fractions of male Fischer rats treated with *Cyclopia intermedia* extract for 10 weeks: Protected cytosolic and microsomal liver fractions of rats against AFB_1-induced mutagenesis Protected cytosolic liver fraction of rats treated with unfermented extract against 2-AAF-induced mutagenesis Microsomal fraction enhanced 2-AAF-induced mutagenesis	Marnewick et al. (2004)
Unfermented; fermented	*In vivo*	4 g leaves/100 ml freshly boiled water, steeped for 30 min	Extract fed (*ad libitum)* to male Fischer 344 rats 1 week after cancer initiated by diethylnitrosamine, followed by cancer promotion by fumonisin B_1 (250 mg/kg) containing diet for 3 weeks marginally inhibited the number and size of DEN-initiated pre-neoplastic liver lesions Fermentation of plant material reduced protective effect of extract Reduction in catalase and GSH:GSSG ratio counteracted by extract Extract of fermented honeybush reduced lipid peroxidation in the liver	Marnewick (2004)
Unfermented; fermented (ethanol/acetone soluble fraction of methanol extract)	*In vivo*	Defatted leaves exhaustively extracted with methanol; dried methanol residue reconstituted in 1:1 ethanol and acetone mixture (30 mg/1 ml)	Ethanol/acetone soluble fraction of a methanol extract (2.07–2.16 mg/mouse) prepared from *Cyclopia intermedia* applied prior to tumor promotor with TPA after initiation with DMBA, inhibited tumour (papilloma) development. Unfermented *Cyclopia intermedia* more effective than fermented counterpart in reducing number and size of papillomas	Marnewick et al. (2005)
Unfermented; fermented	*In vitro*	10 g leaves/100 ml freshly boiled water, steeped for 5 min and freeze-dried	*Cyclopia intermedia*, *Cyclopia subternata*, and *Cyclopia sessiliflora* (0.5–5 mg/plate) inhibited 2-AAF- and AFB_1-induced mutagenesis in the *Salmonella typhimurium* assay (tester strain TA98 and TA100, respectively) in the presence of metabolic activation (S9) Unfermented *Cyclopia genistoides* enhanced mutagenic response of 2-AAF	Van der Merwe et al. (2006)
Unfermented; fermented (water and methanol extracts)	*In vitro*	Water extract—10 g leaves/100 ml freshly boiled water, steeped for 5 min; freeze-dried Methanol extract—defatted leaves extracted twice with methanol (1:2 ratio) for 20 h; evaporated, freeze-dried	Some *Cyclopia genistoides* (West Coast type) and *Cyclopia subternata* water extracts (1.5 μg/ml) bound only to ERβ in whole cell binding assays in COS-1 cells transiently transfected with either ERα or ERβ, while some methanol extracts (1.5 μg/ml) bound to both receptor subtypes with a higher affinity for ERβ *Cyclopia intermedia* and *Cyclopia sessiliflora* water and methanol extracts did not show significant binding to either ER isoform	Verhoog et al. (2007a)

Table 3 (*Continued*)

Processing state	Test system	Extract preparation[a]	Measured effect with details of experimental model	Reference(s)
Unfermented	*In vitro*	Defatted leaves extracted twice with methanol (1:2 ratio) for 20 h; evaporated, freeze-dried	Extracts (2.7×10^{-13} to 7.94×10^{-3} mg/ml) from three harvestings of *Cyclopia genistoides* (West Coast type) tested in different systems: All extracts transactivated an ERE-containing promoter reporter construct via ERβ but not ERα in COS-1 cells transiently transfected with either ERα or ERβ and ERE.vit2.luc with potencies not significantly different from that of genistein Two extracts caused proliferation in MCF-7-BUS cells (shown to be ER dependent through use of ICI 182,780) with potencies significantly lower than E_2 and genistein All extracts antagonised E_2-induced proliferation in MCF-7-BUS cells One extract bound to both ER subtypes in COS-1 cells transiently transfected with either ERα or ERβ with potencies significantly lower than E_2 and genistein. All extracts bound to SHBG from human pregnancy plasma	Verhoog et al. (2007b)
Unfermented; fermented	*In vitro*	10 g leaves/100 ml freshly boiled water, steeped for 5 min; freeze-dried	*Cyclopia intermedia*, *Cyclopia subternata*, *Cyclopia genistoides* and *Cyclopia sessiliflora* (0.02 mg/ml) protected rat liver microsomes against Fe^{2+}-induced lipid peroxidation.	Joubert et al. (2008b)
Unfermented	*In vitro*	Methanol extract—defatted plant material extract thrice with methanol (1:1 ratio) for 30 min each at room temperature; evaporated; freeze-dried Sequential extracts (S)—defatted leaves (16.67 g) sequentially extracted with 3 × 100 ml ethyl acetate, ethanol, methanol, 50% aqueous ethanol and water; evaporated, freeze-dried Non-sequential extracts—defatted leaves (16.67 g) extracted separately at room temperature with 3× 100 ml ethyl acetate, ethanol, methanol, 50% aqueous ethanol and water; evaporated, freeze-dried; "Cup-of-tea" extract (hot)—1.33 g leaves/100 ml freshly boiled water, steeped for 5 min	Methanol extracts from one *C.genistoides* (West Coast type) harvesting and one *Cyclopia subternata* harvesting showed oestrogenic activity in whole cell binding (MCF-7 cells), promoter reporter (ERE-containing promoter reporter stably transfected into T47D-KBlue cells), alkaline phosphatase (in Ishikawa Var1 cells), and E-screen (proliferation in MCF-7-BUS cells) assays Methanol extracts from two other *Cyclopia subternata* harvestings showed no to very little activity in all assays SMet and SEAc had the highest potency and efficacy of all extracts (alkaline phosphatase and E-screen), respectively "Cup-of-tea" extract showed appreciable oestrogenicity (alkaline phosphatase and E-screen) Water extracts prepared at room temperature had no activity (alkaline phosphatase and E-screen) Benchmarking against commercial nutraceuticals (Phytopause Forte, Promesil, Remifemin, and Femolene Ultra) indicated that SMet has comparable potency and SEAc comparable efficacy (whole cell binding, promoter reporter assay, alkaline phoshatase assay, and E-screen)	Mfenyana (2008)
Unfermented	*In vitro*	4 g leaves/100 ml freshly boiled water, steeped for 30 min and freeze-dried	Studies conducted in WCHO5 human oesophageal cancer cells: Unfermented *Cyclopia intermedia* inhibited a higher cytotoxicity than the fermented tea Unfermented extract exhibited similar inhibitory effects on cell proliferation than green tea (*Camellia sinensis*)	Sissing (2008)
Unfermented	*In vivo*	4 g leaves/100 ml freshly boiled water, steeped for 30 min and freeze-dried	*Cyclopia intermedia* extract protected against methylbenzylnitroasamine-induced male Fischer 344 rat oesophageal papillomas by reducing number and size	Sissing (2008)

Abbreviations: E_2: 17β-oestradiol; ER: oestrogen receptor; ERE: oestrogen response element; SHBG: sex-hormone binding globulin; SMet: sequential methanol extract of *Cyclopia subternata*; SEAc: sequential ethyl acetate extract of *Cyclopia subternata*.

[a] Leaves indicate plant material which include varying quantities of stems.

ern Sotho represented the highest percentage of tea drinkers (64%), followed by the Zulu and Swazi (50%). Preparation entails boiling a handful of broken leaves and twigs in approximately 1.5 l of water for 5 min, whereafter the decoction is strained and served, usually without milk, but with sugar added to taste (Rampedi and Olivier, 2005).

Furthermore, the dried twigs are used to make aromatic brooms that are very popular in both rural and urban communities (Rampedi and Olivier, 2005).

3. Botany

The genus *Aspalathus* includes more than 270 species endemic to South Africa. *Aspalathus linearis* (Dahlgren, 1964, 1988) with its needle-like leaves is polymorphic—various wild forms have been described, each with characteristic morphology and geographical distribution. Some forms are prostrate and remain less than 30 cm tall, while other forms grow erect and may reach up to 2 m in height. The size, density of branching, development of short shoots, leaf size and flowering time of the biotypes vary considerably (Dahlgren, 1968). The seeds are hard-shelled, needing scarification to germinate (Kelly and Van Staden, 1985). The biotypes can be divided into either reseeders or resprouters (Schutte et al., 1995). Genetic and polyphenolic differences between various populations have been demonstrated (Van der Bank et al., 1995; Van Heerden et al., 2003).

The red type, currently used for commercial processing, was previously classified as *Aspalathus linearis* (Brum.f) Dahlg. subsp.

linearis (Dahlgren, 1968). Revision by Dahlgren (1988) (as quoted by Van Heerden et al., 2003) led to combination of *Aspalathus linearis* (Brum.f) Dahlg. subsp. *linearis* and *Aspalathus linearis* subsp. *pinifolia* (Wupperthal type) under *Aspalathus linearis*. The red type, also known as the Rocklands type, is divided into the selected and improved Nortier type (cultivated), and the wild-growing Cederberg type, with its broader and coarser leaves (Morton, 1983). *Aspalathus pendula*, closely related to *Aspalathus linearis*, also has needle-like leaves, but it is rarely used for tea manufacture (Van Heerden et al., 2003).

The earliest mention in botanical literature of *Cyclopia* was in 1705, but it was only in 1825 that the plant was identified as *Cyclopia genistoides* (Kies, 1951). More than 20 species of *Cyclopia* have since been described (Kies, 1951; Bond and Goldblatt, 1984; Schutte, 1995). They can be divided according to their fire-survival strategy into reseeders and resprouters.

In natural veld, the bushes normally grow to 1.5 m high, but can reach up to 3 m, depending on the species (Bond and Goldblatt, 1984). Leaf shape and size differ between species—from pubescent, narrow-leafed species (Bond and Goldblatt, 1984) to flat-leafed species (Kies, 1951). The plants have woody stems, a relatively low ratio of leaves to stem, and hard-shelled seeds that germinate poorly if not scarified prior to planting. Trifoliate leaves and flowers with indented calyx are distinctive of the genus. The deep yellow flowers have a characteristic sweet, honey-like aroma, which attracted early herbal tea users. Flowering is usually in spring (September and October) (ARC, 2008).

The genus *Athrixia* contains 14 species, found in southern Africa, tropical Africa and Madagascar, of which nine are endemic to southern Africa (Fox and Young, 1982; Leistner, 2000). *Athrixia phylicoides* is an aromatic, perennial, leafy shrub of up to 1 m with woolly white stems (Fox and Young, 1982). Leaves are simple, alternate, linear to broadly lanceolate, dark-green and shiny below and woolly white above. Flowers are daisy-like with pink to purple petals and bright yellow centers, occurring throughout the year depending on the area (Van Wyk and Gericke, 2000). A related species, *Athrixia elata*, also known as daisy tea or "wildetee" [Afrikaans], is also used as an herbal tea.

4. Geographical distribution

Aspalathus linearis grows naturally in the Cederberg area encompassing the Citrusdal, Clanwilliam and Nieuwoudtville regions, situated in the western parts of the Western Cape Province of South Africa (Dahlgren, 1968). Fig. 1 depicts the rooibos production area, which corresponds with its natural habitat.

Cyclopia species grow fairly localised in the coastal districts and mountainous areas of the Western and Eastern Cape Provinces (Schutte, 1995) (Fig. 1). The major species currently of commercial interest, i.e. *Cyclopia intermedia*, *Cyclopia subternata*, *Cyclopia sessiliflora* and *Cyclopia genistoides*, grow in diverse conditions (Bond and Goldblatt, 1984). The bushes are normally found on the shady and cooler southern slopes of the mountain ranges, except for *Cyclopia genistoides* that is also found on the flats and the sandy coastal areas.

Distribution of *Athrixia phylicoides* is widespread in the mountainous eastern parts of South Africa, including Mpumalanga, Limpopo, the northern parts of the Eastern Cape Province, KwaZulu-Natal, and Swaziland (Fox and Young, 1982) (Fig. 1). The shrub is mainly found in grassland and forest margin scrub areas.

5. Industry

The first rooibos commercial activity was initiated in 1904 by Benjamin Ginsberg when rooibos was marketed under the brand name "Eleven O'Clock" tea. Shortage of Oriental tea during World War II increased the demand for rooibos, but after the war the market collapsed due to the availability of cheap coffees and Oriental tea in more convenient forms. By 1953/54 production of rooibos was uneconomic due to the decrease in demand, overproduction and poor and inconsistent quality. This led to the establishment of the Rooibos Tea Control Board to regulate marketing and ensure quality grading of all tea sold in South Africa (Anon., 1991). This one-channel marketing system was abolished in the mid-1990s. The first exports of 524 tons of rooibos were in 1955, but it is only during the past 14 years that the international market demand steadily grew from 750 tons in 1993 (Anon., 1994) to 7200 tons in 2007. In 2007 total production of rooibos, including unfermented rooibos, was in excess of 14,000 tons, with Germany the major international market (53%), followed by the Netherlands (11%), UK (7%), Japan (6%) and USA (5%) (data supplied by SA Rooibos Council).

Even with its long history of use, honeybush tea never achieved the same popularity as rooibos as an herbal tea and the demand remained sporadic in the early 20th century. No active marketing took place and after World War II consumption of honeybush tea declined to the point where processing was largely discontinued, except for limited activities in the Langkloof area. The first branded product, packaged under the name "Caspa Cyclopia Tea" appeared on the market in the 1960s through the involvement of Benjamin Ginsberg. The commercial success of rooibos led to renewed interest in the 1990s when the National Botanical Institute (Kirstenbosch) and the Agricultural Research Council of South Africa spearheaded projects on commercial cultivation, processing and health-promoting properties to lay the foundation for a formal honeybush industry. The market for honeybush is still very small (<300 tons per annum), but growing. Germany is the major market and the greatest demand is currently for *Cyclopia intermedia* (ARC, 2008).

Athrixia phylicoides has not yet been commercialised, however, a lively informal trade in the tea and brooms is practiced especially in the Gauteng area (Rampedi and Olivier, 2005). Potential for commercialisation is high, as a survey among rural and urban communities in the northern parts of South Africa indicated a willingness to buy *Athrixia phylicoides* products. In order to commercialise the tea, cultivation will be necessary.

5.1. Wild-harvesting and cultivation

Apart from the red type of rooibos, the red-brown, grey and black types were also initially harvested in the wild for tea processing. The flavour of the red-brown type is not very different from that of the red type (Dahlgren, 1968). The flavour and colour of the infusion of the black type was not characteristic of the red type (Coetzee et al., 1953). Marketing of the grey and black types was discontinued in 1966 due to poor quality (Anon., 1967).

The first attempts at rooibos cultivation were in the early 1930s, but it was only after World War II that commercial cultivation started in earnest. Cultivation occurs mainly in the Cederberg mountain region, but extends to areas as far as Darling and Nieuwoudtville. Seedlings are used to propagate the plants, leading to large phenological and genetic variation in the cultivated plants.

Planting of seedlings takes place between June and August and approximately 8 months after planting, the plants are topped to a height of 30 cm to stimulate branching. The first harvest takes place during the second summer, but full production is only realised after

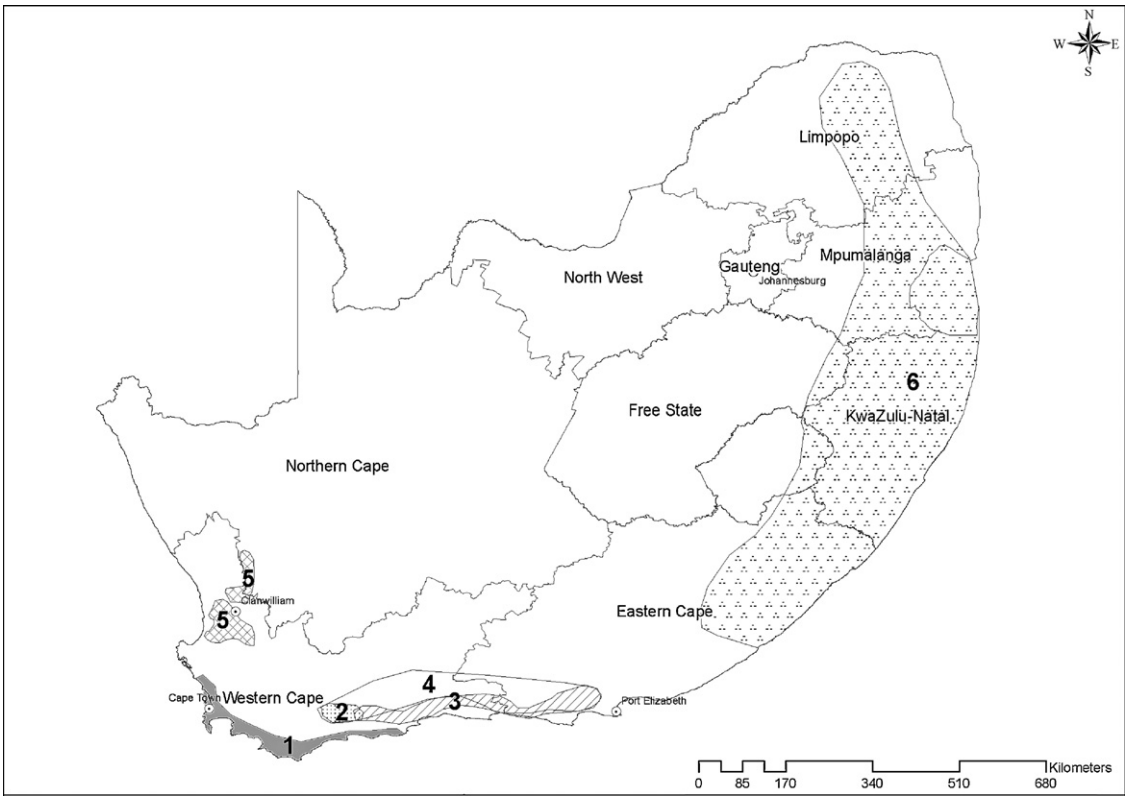


Fig. 1. Production area of *Aspalathus linearis* and natural distribution of *Cyclopia* species and *Athrixia phylicoides*. Key: (1) *Cyclopia genistoides*; (2) *Cyclopia sessiliflora*; (3) *Cyclopia subternata*; (4) *Cyclopia intermedia*; (5) *Aspalathus linearis*; (6) *Athrixia phylicoides* (distribution data of *Athrixia phylicoides* obtained from Rampedi and Olivier, 2005).

3 years. Whereas earlier plantations had a commercial lifespan of 7 years or longer (Cheney and Scholtz, 1963), problems with die-back severely limit the current lifespan of the plants (Joubert and Schulz, 2006).

Propagation and cultivation trials by Dr Hannes de Lange of the National Botanical Institute of South Africa (Kirstenbosch) in the early 1990s led to interest in commercial production of honeybush. After these initial trials, evaluating different *Cyclopia* species at different areas and locations in the Western and Eastern Cape Provinces of South Africa, *Cyclopia subternata*, a reseeder, and *Cyclopia genistoides*, a resprouter, were selected for commercial cultivation. Either seedlings or cuttings are used for propagation. More than 200 hectares are currently under cultivation in fairly localised areas spanning from the Overberg in the southern part of the Western Cape Province to the Langkloof in the Eastern Cape Province. *Cyclopia intermedia* is not cultivated, since it grows very slowly and cannot be harvested annually. Although small, the industry is supported by an active horticultural research program, involving investigation of nutrient requirements, soil preparation techniques, harvesting practices and plant improvement (selections and cross-breeding), as well as product research (ARC, 2008).

Currently, *Athrixia phylicoides* is only harvested in the wild. Recently, signs of over-exploitation have been noted due to unscrupulous plant collectors practicing unsustainable harvesting techniques, i.e. uprooting of the whole plant (Rampedi and Olivier, 2005). These are normally traders from the urban areas who come to rural areas to harvest plants for brooms or tea. Local, rural users normally practice sustainable harvesting techniques, namely picking only branches. The current over-exploitation indicates a great demand for the plant, but sustainable harvesting and/or cultivation will be needed to successfully commercialise *Athrixia phylicoides* products.

Some preliminary work has been done to investigate propagation and cultivation of *Athrixia phylicoides* (Araya, 2005; Mudau et al., 2005, 2006). Propagation using seeds or cuttings is possible (Araya, 2005).

5.2. Agroprocessing

5.2.1. Tea manufacture

Rooibos is harvested during the hot summer months and early autumn (January to April) by topping the whole bush to ca. 45 cm. The active growth should not be more than 50 cm, otherwise the plant material will produce a weak tea and no flowers should be present, as they are detrimental to quality. Leaves become red-brown when the shredded plant material is "fermented" in heaps at ambient temperature, usually for 12–14 h, before being sun-dried. The temperature of the heap naturally increases during fermentation and should preferably be between 38 and 42 °C (Joubert and De Villiers, 1997). Fermentation time depends on factors such as the presence of young growth, the age of the bush and cultivation area (Joubert, 1994). Shredding of the shoots initiates enzymatic oxidation of the polyphenols, leading to rapid browning (Joubert, 1996). Wetting of the heap, followed by bruising is, however, necessary to accelerate the "fermentation" process. Polyphenols released during bruising is absorbed by the shredded stems, colouring it red-brown to give a more uniformly coloured product. The fermentation heap is turned over several times to ensure adequate aeration required for oxidation (Joubert, 1994). Poor aeration results in a low quality product (Joubert, 1998). As soon as the characteristic sweet aroma has formed, the fermentation heap is spread open in the sun to dry. Sieving of the dried tea and steam pasteurisation to ensure a product of high microbial quality are done before the tea is packed (Joubert and Schulz, 2006).

The first commercial green rooibos (it will be referred to as unfermented rooibos in the remaining text and tables) was produced in 2001 to meet a demand by the international market for a product with a higher antioxidant activity than the traditional "fermented" tea (De Beer and Joubert, 2002). To retain the green leaf colour, oxidative changes should be kept to a minimum. High-end quality products can be produced either by drying of shredded plant material without delay under vacuum, drying of whole shoots to a critical moisture content before shredding or steaming of the fresh shoots to inactivate enzymes before shredding (unpublished data). Mostly, unfermented rooibos is produced by spreading the shredded plant material in a thin layer in the sun for quick drying. This leads to loss in aspalathin content, and if not properly dried, to slow browning. Unfermented rooibos currently comprises 1% of total sales on the global market (data supplied by SA Rooibos Council).

Early descriptions of the use of *Cyclopia* species as a "bush tea" stated that the leaves and flowers were sun-dried, without indication that they were first "sweated" or "fermented" (Hofmeyer and Phillips, 1922). Later practice was to heat the leaves in an oven for 1 h, whereafter the plant material was sun-dried (Hofmeyer and Phillips, 1922). According to Marloth (1909, 1925) the leaves and twigs, harvested during the flowering period, were piled into large fermentation heaps for several days, followed by sun-drying. Spontaneous heat generation and oxidation resulted in the development of the sought-after sweet, honey-like flavour and dark brown colour. However, the prolonged period of "heap fermentation" at temperatures ranging from ambient on the outside to ca. 60 °C in the middle of the heap (Du Toit et al., 1998) is accompanied by extensive mould and bacterial growth (Du Toit et al., 1999), giving a product of poor microbial quality. Since the traditional heap fermentation method allowed for very little control over the production process, and faced with the demand for high quality tea, as well as export regulations requiring very low levels of microbial contaminants, heap fermentation became obsolete and alternatives had to be found. Studies by Du Toit and Joubert (1998a, 1999) showed that high temperature "fermentation" and drying under controlled conditions ensured tea of high sensory quality. Fermentation at ≥60 °C was also found adequate to inhibit growth of thermophilic moulds (Du Toit et al., 1999). High temperature fermentation necessitates adding of water before fermentation. This not only improves the uniformity of the colour of the dried product, but also improves the release of the tea solids when preparing the infusion (Du Toit and Joubert, 1998b). Contrary to early popular belief, the presence of flowers is not essential for the characteristic sweet, honey-like flavour of the tea, although their presence does improve the flavour (Du Toit and Joubert, 1999).

Current practice is to harvest the plant material in summer to late autumn before flowering can occur, as flowering places the plant under stress. Batch rotary fermentation, a concept developed initially for rooibos fermentation (Joubert and Müller, 1997), but adopted by the honeybush industry, takes place at 70 °C for 60 h or 80–85 °C for 18 h. Drying either takes place in a rotary drier under controlled conditions or in the sun on drying racks.

Commercial production of unfermented honeybush followed soon after production of unfermented rooibos commenced. Although a vacuum drying process of steamed plant material was patented (De Beer and Joubert, 2002), normal hot air drying of the steamed, shredded plant material was found to adequately retain the green colour of the tea leaves. Maicu (2008) showed that steaming directly after maceration improved the colour retention of unfermented *Cyclopia subternata*. Good stability was also shown for mangiferin and hesperidin content during manufacture and storage of unfermented *Cyclopia subternata*.

Branches of *Athrixia phylicoides* are harvested and sun-dried. The fine twigs and leaves are then removed for use as a tea beverage, while the stripped branches are tied together to make brooms. Cutting of the plant material before or after drying will need to be investigated for commercial production. The woolly hairs on the leaves and stems form a sticky residue on the cutting blades of a normal tea cutter even after sun-drying, which impedes further cutting (Jana Olivier, UNISA, 2006; personal communication). Possible microbial contamination and pasteurisation will also need attention before a commercial product can be marketed.

5.2.2. Extract preparation

Research was carried out in the early 1980s on the development of an instant rooibos to provide the consumer with rooibos in a more convenient form (Joubert, 1984; Joubert, 1988a) as brewing of a strong cup of tea was a time-consuming process. The flavour of the reconstituted powder was affected, although no preference for the freshly brewed tea was found. Clarification, which entailed removal of the precipitate that formed upon concentration and cooling, markedly decreased the polyphenol content of the dried extract (Joubert, 1990a). The concept of a soluble rooibos product only found commercial application in 2000 in South Africa with the production of powdered extracts for the beverage, food and dietary supplement markets (Anon., 2005a,b), while an instant rooibos product for food applications was released in May 2008 (at the Vitafoods Exhibition in Geneva, Switzerland).

Higher temperatures, higher water-to-leaf ratios and longer extraction times favour extraction of soluble solids and polyphenols from rooibos (Joubert, 1988b, 1990b,c; Joubert and Hansmann, 1990). The yield of soluble solids and polyphenols from fermented rooibos during extraction can be increased by pretreating the plant material with a cocktail of hydrolysing enzymes expressed by food grade fungi (Pengilly et al., 2008).

Skin-care products were the first non-beverage application of rooibos extracts. In South Africa fermented rooibos was used for these products. Antioxidant research showing unfermented rooibos to contain higher levels of antioxidants (Von Gadow et al., 1997a) sparked interest in unfermented rooibos as source material for extract production for the international cosmetic market (Tiedtke and Marks, 2002; Otto et al., 2003a,b).

Powdered honeybush extract, as antioxidant, finds similar applications to rooibos, although the market is less familiar with the product, largely due to fact that the honeybush industry is still small and was only recently revived.

Industrial production of an unfermented *Cyclopia sessiliflora* extract was investigated (Grüner et al., 2003). *Cyclopia genistoides* is preferred for production of mangiferin-enriched extracts. Waste material, comprising coarse plant material with a high stem content, not suitable for selling in loose or tea bag format, is often used for preparation of extracts.

No extract manufacturing is currently done with *Athrixia phylicoides*. Studies on the polyphenolic content and antioxidant activity of *Athrixia phylicoides* extracts indicate that this is a potential source of antioxidant-rich extracts for the nutraceutical and cosmeceutical industry (De Beer and Joubert, 2007; McGaw et al., 2007).

5.3. Quality control

Regulatory quality control of rooibos and honeybush is limited to pesticide residues and microbial contamination. Tea manufacturers each exercise their own set of quality standards in terms of cut size, colour and flavour. The use of objective colour measurement for predicting quality of rooibos tea infusions was evaluated (Joubert, 1995). The red colour component of infusions was shown to be important in visual grading, i.e. infusion with a higher

colour grading had higher a^* values (according to CIELab system). No specifications exist for polyphenolic content and antioxidant activity, in spite of their associated importance in the health-promoting properties of rooibos and honeybush. Aspalathin, due to its instability under poor processing conditions, could be a good quality parameter for unfermented rooibos (Schulz et al., 2003).

A major extract manufacturer in South Africa uses total polyphenol (TP) content and total antioxidant activity (TAA) to standardise their extracts. A good correlation ($r = 0.99$) exists between TAA and TP content of unfermented rooibos extracts (Joubert et al., 2008a). The TP content of unfermented *Cyclopia genistoides* extracts is, however, not a good indicator of TAA ($r = 0.27$) (Joubert et al., 2008a), but a moderate correlation ($r = 0.85$) was obtained for *Cyclopia subternata* (Maicu, 2008). The TAA of unfermented rooibos extracts correlates with their aspalathin content, while TAA correlates moderately and poorly with the mangiferin content of unfermented *Cyclopia genistoides* (Joubert et al., 2008a) and *Cyclopia subternata* (Maicu, 2008), respectively. A German producer of enriched extracts from unfermented rooibos and honeybush standardises on aspalathin and mangiferin content, respectively (Bernd Weinreich, Raps Foundation, Germany, 2005; personal communication).

Progress has been made in the development of rapid analytical techniques to determine the major polyphenolics of rooibos and honeybush (*Cyclopia genistoides* and *Cyclopia subternata*) in the dried, unfermented plant material for quality control purposes. Near infrared spectroscopy (NIRS) is suitable for quantification of the aspalathin (Schulz et al., 2003; Manley et al., 2006) and dihydrochalcone content of unfermented rooibos, while the NIRS calibration model developed for nothofagin is suitable for screening purposes (Manley et al., 2006). FT-Raman spectroscopy can also be used for quantification of aspalathin, nothofagin and dihydrochalcone content of the plant material (Baranska et al., 2006). NIRS calibration models for the prediction of mangiferin and hesperidin content of unfermented *Cyclopia genistoides* (Joubert et al., 2006a) and mangiferin and xanthones content in unfermented *Cyclopia subternata* (Maicu, 2008) are adequate for screening purposes.

Transmittance NIRS calibration models, developed for aspalathin, TP and soluble solids content, as well as TAA of unfermented rooibos water extracts, were found to give poor accuracy, probably due to the low concentration of compounds and soluble solids in solution (Manley et al., 2006). Other methods, suitable for adaptation to microplate format for high throughput, especially in cases where large numbers of samples need to be screened, such as in a selection and breeding program, were thus investigated. The dihydrochalcone content of extracts of unfermented rooibos as determined spectrophotometrically at 288 nm correlates highly ($r = 0.98$) with the sum of the aspalathin and nothofagin contents of the extracts as determined by HPLC (Joubert et al., 2008b). A colorimetric method, using $AlCl_3$, as alternative to HPLC could be used for determination of the mangiferin content of methanol extracts of unfermented *Cyclopia genistoides* (Joubert et al., 2008a). Mangiferin and xanthone content of hot water extracts of unfermented *Cyclopia subternata* could also be successfully predicted, using the $AlCl_3$ method (Maicu, 2008).

Botha (2005), using principal component analysis and linear discriminant analysis of NIR spectral data, showed that NIRS could be used to differentiate clearly between unfermented rooibos and unfermented *Cyclopia genistoides* plant material. It can thus be used by processors in cases where milled plant material needs to be identified.

HPLC, however, should remain an important tool in ensuring product authenticity. Aspalathin, unique to rooibos, provides the analyst with a very specific marker. Indiscriminate harvesting from the wild, especially to meet the demand for organic tea, can result in tea on the market with atypical polyphenolic profiles. Fresh plant material sampled at a processor was shown to contain no aspalathin (Joubert and Schulz, 2006). This is not unexpected as Van Heerden et al. (2003) reported that some wild types of *Aspalathus linearis* contain no aspalathin.

6. Chemical composition

Although rooibos does not contain caffeiene, traces of the alkaloid sparteine were found by Van Wyk and Verdoorn (1989).

It is considered a low tannin beverage (Reynecke et al., 1949; Blommaert and Steenkamp, 1978), especially when compared to *Camellia sinensis* teas. The leaf tannin content of fermented rooibos is 3.2% (Reynecke et al., 1949) to 4.4% (Blommaert and Steenkamp, 1978). Studies carried out at the Department of Chemistry of the University of the Free State (Bloemfontein, South Africa), however, showed that the dried water extract of fermented rooibos consists of as much as 50% complex tannin-like substances, whereas a methanol extract of unfermented rooibos contains 14% tannin. Little structural information is, however, available, but electron-spray ionisation mass spectrometry analysis indicated a pentamer (unpublished data). Cleavage of the isolated polymer in an acidic medium in the presence of phloroglucinol indicated an irregular procyanidin type heteropolymer, containing (+)-catechin and (−)-epicatechin chain extending units and (+)-catechin as terminal unit (Marais et al., 1998). The dimer, procyanindin B3, and the trimer, bis-fisetinidol-(4β,6:4β,8)-catechin, are present in very low concentrations in fermented rooibos (Ferreira et al., 1995) (Table 4).

The monomeric flavonoid composition of rooibos is unique in that it contains aspalathin, a C–C linked dihydrochalcone glucoside (Koeppen and Roux, 1965a, 1966; Rabe et al., 1994) and the cyclic dihydrochalcone, aspalalinin (Shimamura et al., 2006), to date isolated only from *Aspalathus linearis*. Nothofagin, the 3-dehydroxy dihydrochalcone glucoside, is another rare compound, previously shown to be present in the heartwood of *Nothofagus fusca* (Hillis and Inoue, 1967). Rooibos also contains several other C–C linked β-D-glucopyranosides, i.e. the flavones orientin, iso-orientin (Koeppen and Roux, 1965b), vitexin and isovitexin (Rabe et al., 1994), and the flavanones, dihydro-orientin, dihydro-iso-orientin (Bramati et al., 2002) and hemiphlorin (Shimamura et al., 2006).

Koeppen and Roux (1965a) showed photochemical conversion of aspalathin to dihydro-iso-orientin (eriodictyol-6-*C*-β-D-glucopyranoside) and dihydro-orientin (eriodictyol-8-*C*-β-D-glucopyranoside) and the formation of polymeric substances in an ethanolic solution exposed to sunlight and oxygen. Dihydro-iso-orientin formed preferentially with traces of dihydro-orientin, although its concentration increased with time, considered to result from the conversion of dihydro-iso-orientin. Marais et al. (2000) confirmed the oxidative conversion of aspalathin, in the presence of light and heat (30 °C), into the diastereomeric flavanone mixture, (*S*)- and (*R*)-eriodictyol-6-*C*-β-D-glucopyranoside. Krafczyk and Glomb (2008) provided further insights into the chemical pathways pertaining to conversion of aspalathin under oxidative conditions. Incubation at 37 °C in a phosphate buffer (pH 7.4) led to degradation of aspalathin with the initial formation of the diastereomeric mixture of dihydro-iso-orientin as major products, together with the diastereomeric mixture of dihydro-orientin as minor products in a stable ratio. Maximum iso-orientin and orientin concentrations occurred after 6 h. Degradation of iso-orientin was relatively slow, while no orientin was detectable after 10 h. Irreversible conversion of iso-orientin to orientin also took place, but it was a minor product among uncharacterized brown material. The latter gave no discrete signals detectable using HPLC with diode-array detection.

Table 4
Secondary metabolites identified in fermented *Aspalathus linearis* plant material

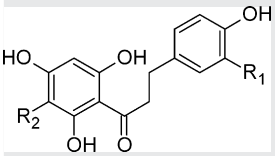
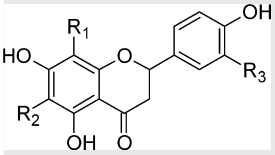
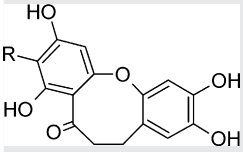
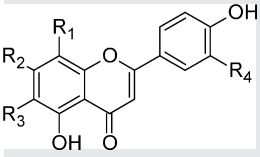
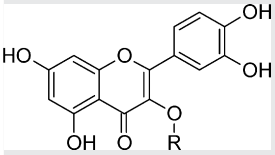
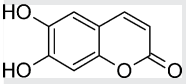
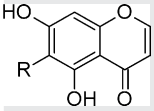
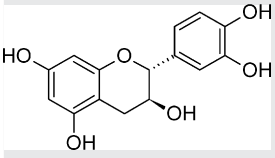
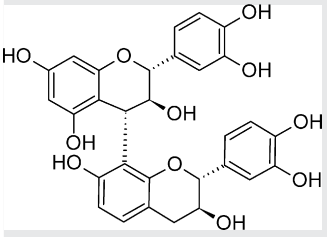
General structure	Compound type, names and substituents
	Dihydrochalcone Aspalathin[a,b,c,d,f,g,k]: R_1 = OH, R_2 = *C*-β-D-glucosyl Nothofagin[e,f,g,k]: R_1 = H, R_2 = *C*-β-D-glucosyl
	Flavanone Dihydro-orientin [(*R*)/(*S*)-eriodictyol-8-glucoside][f,g,k]: R_1 = *C*-β-D-glucosyl, R_2 = H, R_3 = OH Dihydro-*iso*-orientin [(*R*)/(*S*)-eriodictyol-6-glucoside][f,g,k]: R_1 = H, R_2 = *C*-β-D-glucosyl, R_3 = OH Hemiphlorin[g]: R_1 = *C*-β-D-glucosyl, R_2 = R_3 = H
	Cyclic dihydrochalcone Aspalalinin[g]: R = *C*-β-D-glucosyl
	Flavone Orientin[a,c,f,g,h,i,k]: R_1 = *C*-β-D-glucosyl, R_2 = R_4 = OH, R_3 = H *iso*-orientin[c,f,h,i,k]: R_1 = H, R_2 = R_4 = OH, R_3 = *C*-β-D-glucosyl Vitexin[a,c,f,g,k]: R_1 = *C*-β-D-glucosyl, R_2 = OH, R_3 = R_4 = H Isovitexin[c,f,g,k]: R_1 = R_4 = H, R_2 = OH, R_3 = *C*-β-D-glucosyl Luteolin[c,f,g,k]: R_1 = R_3 = H, R_2 = R_4 = OH Luteolin-7-*O*-glucoside[d]: R_1 = R_3 = H, R_2 = *O*-β-D-glucosyl, R_4 = OH Chrysoeriol[c,f,k]: R_1 = R_3 = H, R_2 = OH, R_4 = OCH_3
	Flavonol Quercetin[c,f,g,k]: R = H Isoquercitrin[c,f,g,i,k]: R = *O*-β-D-glucosyl Hyperoside[f,g,k]: R = *O*-β-D-galactosyl Rutin[f,i,k]: R = *O*-β-D-rutinosyl Quercetin-3-*O*-β-D-robinoside[g]: R = *O*-robinosyl
	Coumarin Esculetin[g]
	Chromone 5,7-Dihydroxy-6-*C*-glucosyl-chromone[d]
	Monomeric flavan-3-ol (+)-Catechin[d,k]
	Oligomeric flavan-3-ol Procyanidin B3[d]

Table 4 (*Continued*)

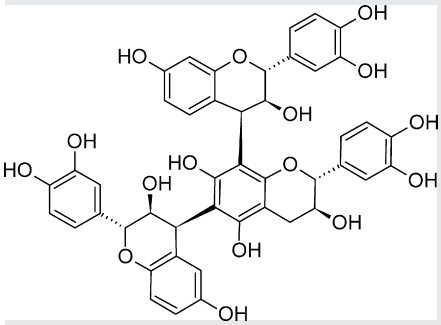
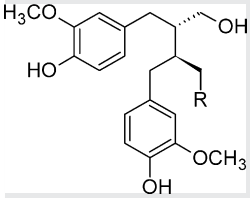
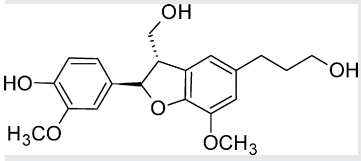
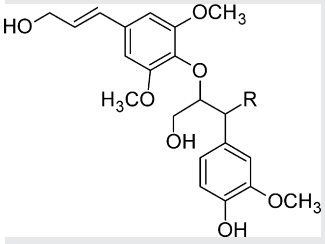
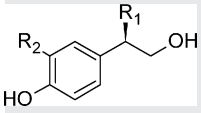
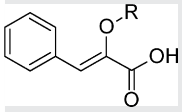
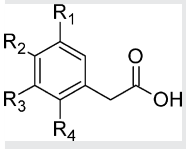
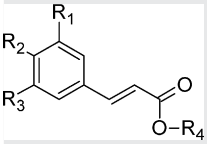
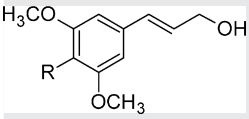
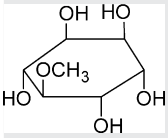
General structure	Compound type, names and substituents
	Oligomeric flavan-3-ol Bis-fisetinidol-(4β,6:4β,8)-catechin[d]
	Lignan Secoisolariciresinol[g]: R = OH Secoisolariciresinol-*O*-glucoside[g]: R = *O*-glucosyl
	Lignan Vladinol F[g]
	Lignan 3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxy-1-(*E*)-propenyl)-2,6-dimethoxyphenoxy] propyl-β-D-glucopyranoside[g]: R = *O*-β-D-glucosyl
	Glycol derivative *p*-Hydroxyphenylglycol[g]: $R_1 = R_2 = H$ Vanylglycol[g]: R_1 = OH, $R_2 = OCH_3$
	Phenylpyruvic acid derivative 3-Phenyl-2-glucopyranosyloxypropenoic acid[j]: R = *O*-glucosyl
	Phenolic carboxylic acid *p*-Hydroxybenzoic acid[c,g,k]: $R_1 = R_3 = H$; R_2 = OH; $R_4 = H$ Protocatechuic acid[c,k]: $R_1 = R_2$ = OH, $R_3 = R_4 = H$ 3,5-Dihydroxybenzoic acid[k]: $R_1 = R_3 = H$; $R_2 = R_4$ = OH Gentisic acid[k]: $R_1 = R_3$ = OH; $R_2 = R_4 = H$ Salicylic acid[k]: $R_1 = R_2 = R_3 = H$; R_4 = OH Gallic acid[k]: $R_1 = R_2$ = OH, R_3 = OH; $R_4 = H$ Vanillic acid[c,k]: $R_1 = OCH_3$,; R_2 = OH; $R_3 = R_4 = H$ Syringic acid[d,k]: $R_1 = R_3 = OCH_3$; R_2 = OH; $R_4 = H$

Table 4 (*Continued*)

General structure	Compound type, names and substituents
	Hydroxycinnamic acid and derivative 3,4,5-Trihydroxycinnamic acid[c]: $R_1 = R_2 = OH$; $R_4 = H$ *p*-Coumaric acid[c,g,k]: $R_1 = R_3 = H$, $R_2 = OH$; $R_4 = H$ Caffeic acid[c,k]: $R_1 = R_2 = OH$, $R_3 = H$; $R_4 = H$ Ferulic acid[c,k]: $R_1 = OCH_3$, $R_2 = OH$, $R_3 = H$; $R_4 = H$ Sinapic acid[c]: $R_1 = R_3 = OCH_3$, $R_2 = OH$; $R_4 = H$ Chlorogenic acid[k]: $R_1 = R_2 = OH$, $R_3 = H$; R_4 = quinic acid
	Aldehyde Syringin[g]: R = *O*-glucosyl
	Inositol (+)-Pinitol[d]

[a] Koeppen and Roux (1965a); identification by NMR.
[b] Koeppen and Roux (1966); identification by NMR.
[c] Rabe et al. (1994); identification by NMR.
[d] Ferreira et al. (1995); identification by NMR.
[e] Joubert (1996); identification by co-elution with pure standard.
[f] Bramati et al. (2002); identification by LC–MS.
[g] Shimamura et al. (2006); identification by NMR.
[h] Koeppen and Roux (1965b); identification by NMR.
[i] Koeppen et al. (1962); identification by NMR.
[j] Marais et al. (1996); identification by NMR.
[k] Krafczyk and Glomb (2008).

Oxidative conditions such as those prevalent during the fermentation process could lead to the conversion of aspalathin to its corresponding flavanones, as indicated by the appearance of peaks, co-eluting with dihydro-iso-orientin, during fermentation of rooibos (Joubert, 1996). The novel compound, 5,7-dihydroxy-6-*C*-β-D-glucopyranosyl chromone, possibly forms from dihydro-iso-orientin during fermentation (Ferreira et al., 1995). According to Hillis and Inoue (1967) nothofagin failed to convert to the corresponding flavanone under conditions similar to those used by Koeppen and Roux (1965a).

Other flavones present in rooibos include chrysoeriol, luteolin and luteolin-7-*O*-glucoside, while the flavonols are quercetin and its O-linked glycosides, quercetin-3-robinobioside, hyperoside, isoquercitrin and rutin (Snyckers and Salemi, 1974; Rabe et al., 1994; Bramati et al., 2002; Kazuno et al., 2005; Shimamura et al., 2006). Phenolic acids, lignans (vladinol F, secoisolariciresinol and a secoisolariciresinol glucoside) and the coumarin, esculetin, are also present (Rabe et al., 1994; Shimamura et al., 2006; Krafczyk and Glomb, 2008).

Quantitative data for rooibos flavonoids deal only with the major constituents. Aspalathin is the major flavonoid of unfermented rooibos (Table 5) and it remains one of the major constituents of the water extract of fermented rooibos (Bramati et al., 2002), despite its substantial decrease during fermentation (Joubert, 1996). Other major compounds are orientin and iso-orientin. Trace quantities of the aglycones, luteolin, quercetin and chrysoeriol are present (Toyoda et al., 1997). Hot water extracts of unfermented and fermented rooibos contain 15.22 and 2.96% flavonoids, respectively (Joubert et al., 2005). Extracts of unfermented and fermented rooibos analysed by Bramati et al. (2003) contained only 5.9 and 0.55% flavonoids, respectively. The TP content of the hot water extract of rooibos decreases with fermentation (Standley et al., 2001; Joubert et al., 2008b). Powdered rooibos extract, prepared from waste material, contains low levels of orientin, iso-orientin and isovitexin (Table 5).

Joubert and Schulz (2006) demonstrated large variations in the aspalathin and nothofagin content of 97 samples of unfermented rooibos, harvested from 24 plantations. The aspalathin and nothofagin content of bushes within the same plantation also varies. This is attributed to genetic variation due to the use of seeds for propagation. Fermentation will further aggravate variation in the dihydrochalcone content of fermented rooibos, since the process takes place in the open air under uncontrolled conditions. Fermented rooibos contains between 0.02 and 1.16% aspalathin and between 0 and 0.4% nothofagin (Joubert and Schulz, 2006).

Erroneously, a high ascorbic acid content is associated with fermented rooibos (Morton, 1983), a "fact" that has since been repeated in some popular and scientific publications dealing with rooibos. Not only is ascorbic acid unstable under oxidative conditions such as prevalent during the fermentation process, but its survival during the long fermentation period is highly unlikely. For the analysis done by Hesseling et al. (1979), a non-specific method, detecting all carbonyl groups (Brady's reagent; 2,4-dinitrophenylhydrazine) after oxidation, was used. This method in fact measured the carbonyl groups of the oxidised polyphenolic compounds. The apparent increase in "ascorbic acid" with increasing infusion time as noted by Hesseling et al. (1979) is also attributed to enhanced extraction of rooibos polyphenolic compounds with time (Joubert, 1990c; Jaganyi and Wheeler, 2003). Testing of fresh, unfermented rooibos at ARC Infruitec-Nietvoorbij, using a very specific enzymatic method for ascorbic acid, did not indicate its presence (unpublished data).

The mineral and fluoride content of fermented rooibos and infusions are summarised in Table 6. The highest concentrations were

Table 5
Total polyphenol[a] and major flavonoid[b] content of *Aspalathus linearis* hot water extract

Compound	Unfermented rooibos[c]	Fermented rooibos[c]	Fermented rooibos (industrial extract)[d]
Aspalathin	3.49–8.31	0.61–0.72	0.35
Nothofagin	nd[c], 1.08[e]	nd[c], 0.17[e]	0.11
Orientin	0.40–0.68	0.61–0.76	0.16
iso-orientin	0.47–0.78	0.74–0.94	0.05
Vitexin	0.05–0.07 (0.13)[e]	nd, 0.11	0.24
Isovitexin	<0.02 (0.17)[e]	0.21–0.24	0.09
Isoquercitrin + rutin	<0.02 (0.16)[e]	0.24–0.35	0.32
Luteolin	nd[c]	nd[c], trace[e]	Trace
Quercetin	<0.02	0.01–0.02	Trace
Chrysoeriol	nd[c]	nd[c], trace[e]	Trace
Total polyphenols	35.08[f]–39.30[e]	29.69[f]–34.25[e]	27.3

[a] Results expressed as g gallic acid equivalents/100 g dried extract determined with Folin–Ciocalteu reagent.
[b] mg compound/g dried extract.
[c] Van der Merwe (2004) except when stated otherwise. Six samples were analysed. nd: not detected.
[d] Joubert and Schulz (2006). Waste material ($n = 9$) containing a large percentage of stems was used for extract preparation.
[e] Joubert et al. (2005). One sample was analysed.
[f] Joubert et al. (2008b). Six samples were analysed.

obtained for Na and K, followed by Mg, Ca and P. The 5-min infusion contains only traces of iron, while exhaustive extraction increased the iron concentration to 0.15 μg/ml. According to data reported by Morton (1983) a cup of rooibos tea contains 2.8 mg iron, 18 mg calcium, 28 mg mangesium, 10 mg phosphate and 47 mg potassium.

The volatile fraction of fermented rooibos was characterised by Habu et al. (1985) and Kawakami et al. (1993). Guaicol, dihydroactinidiolide, β-ionone, 5,6-epoxy-β-ionone and benzaldehyde are some of the major constituents of the vacuum steam distillate (Habu et al., 1985).

Similar to rooibos, honeybush is caffeine-free (Greenish, 1881) and has a low tannin content (Marloth, 1925; Terblanche, 1982). Approximately 30% of the TP content and 4.34% of the hot water soluble solids of fermented *Cyclopia maculata*, is tannin (Du Toit and Joubert, 1998b). The tannin is of the proanthocyanindin type (Marnewick et al., 2005).

In-depth investigation of the polyphenolic composition of *Cyclopia* spp. is limited to *Cyclopia intermedia* (fermented) and *Cyclopia subternata* (unfermented) (Ferreira et al., 1998; Kamara et al., 2003, 2004). However, some polyphenols were also identified by LC–MS in *Cyclopia genistoides* and *Cyclopia sessiliflora* by Joubert et al. (2008b). Table 7 summarises the compounds present in *Cyclopia*. The xanthones, mangiferin and isomangiferin, and the flavanone, hesperidin, are present in all species analysed to date. De Nysschen et al. (1996) also showed the ubiquitous presence of hesperetin and isosakuranetin in a number of species, including *Cyclopia intermedia* and *Cyclopia subternata*. No detectable quantities of isosakuranetin were, however, found in several extracts each of *Cyclopia intermedia*, *Cyclopia subternata*, *Cyclopia sessiliflora* and *Cyclopia genistoides* using LC–MS (Joubert et al., 2008b). *Cyclopia intermedia* contains a number of flavanones, flavones, flavonols, isoflavones and coumestans (Ferreira et al., 1998; Kamara et al., 2003). The polyphenolic composition of *Cyclopia subternata* differs greatly from that of *Cyclopia intermedia*. No coumestans were found in *Cyclopia subternata*.

Methanol extracts of unfermented *Cyclopia intermedia*, *Cyclopia genistoides* (West Coast type), *Cyclopia sessiliflora* and *Cyclopia maculata* (ex Genadendal), analysed for the major compounds, i.e. mangiferin, isomangiferin and hesperidin, showed that *Cyclopia genistoides* contained the highest concentrations of mangiferin (3.61%) and isomangiferin (0.54%), whereas *Cyclopia intermedia* contained the highest concentration of hesperidin (1.76%) (Joubert et al., 2003) (Table 8). Quantitative differences in the polyphenolic composition of two types of *Cyclopia genistoides*, i.e. Overberg and West Coast, exist. The Overberg type contains significantly more mangiferin, but less hesperidin than the West Coast type, while the isomangiferin content is not significantly different between the two types (Joubert et al., 2003). Hot water extracts of another set of plant material comprising a number of batches each of unfermented *Cyclopia genistoides*, *Cyclopia sessiliflora*, *Cyclopia intermedia* and *Cyclopia subternata* contained 10.04, 4.39, 2.79 and 1.19% mangiferin, respectively (Joubert et al., 2008b). No isomangiferin was detected in the *Cyclopia intermedia* extract, whereas the extracts of *Cyclopia genistoides* and *Cyclopia intermedia* contained the highest isomangiferin (1.73%) and hesperidin (1.12%) contents, respectively.

The TP content of the hot water extract decreases with fermentation of the plant material and varies between species. Du Toit and Joubert (1999) showed that *Cyclopia intermedia* and *Cyclopia maculata* (ex Du Toit's Kloof mountains) retained 75–76% of its water extractable TP content during fermentation at 70 °C for 60 h. Using the same fermentation conditions, Hubbe (2000) obtained substantially lower values for *Cyclopia maculata* (ex Genadendal), and *Cyclopia intermedia,* retaining 53 and 57%, respectively. *Cyclopia subternata*, *Cyclopia genistoides* and *Cyclopia sessiliflora* retained, respectively, 53, 67 and 71%. Using plant material orig-

Table 6
Mineral and fluoride content of fermented *Aspalathus linearis* plant material (μg/g) and infusions (μg/ml)

Mineral	Leaves[a]	Leaves[b]	5 min infusion[b]	60 min infusion[b]
Al	98 ± 3	99 ± 13	0.05 ± 0.03	0.20 ± 0.07
B		30 ± 6	0.26 ± 0.07	0.44 ± 0.09
Ca	2130 ± 30	2017 ± 276	6.35 ± 1.69	10.06 ± 1.75
Co		<0.05	Trace	Trace
Cr		0.61 ± 0.22	Trace	Trace
Cu		2.26 ± 1.01	0.07 ± 0.04	0.14 ± 0.02
Fe		90 ± 12	<0.02	0.15 ± 0.04
K		4083 ± 944	58.63 ± 17.72	68.76 ± 13.84
Mg	2360 ± 35	1531 ± 156	10.17 ± 1.72	16.98 ± 1.84
Mn	70 ± 1	83 ± 19	0.40 ± 0.11	0.68 ± 0.16
Na		2467 ± 184	43.33 ± 6.95	51.88 ± 4.52
P		679 ± 127	7.57 ± 1.97	10.49 ± 1.90
Zn	12 ± 0.3	11 ± 3	0.08 ± 0.02	0.10 ± 0.02
Fluoride[c]			1.29 ± 0.27	

[a] Mokgalaka et al. (2004).
[b] Unpublished results. Analysis by Analytical Laboratory, ARC Infruitec-Nietvoorbij, Stellenbosch using ICP-AES. Ten samples were analysed. The infusions were prepared by steeping 2 g tea in 100 ml water freshly boiled water for 5 min, or by refluxing for 60 min.
[c] Touyz and Smit (1982). The infusion was prepared by steeping 1.5 g tea in 100 ml freshly boiled water.

Table 7
Secondary metabolites identified in plant material of *Cyclopia* species

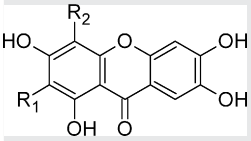
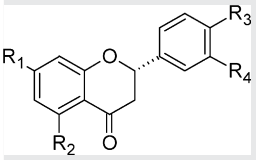
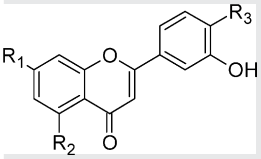
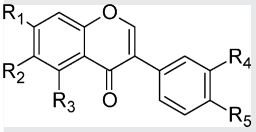
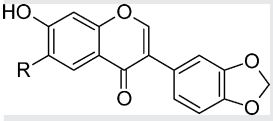
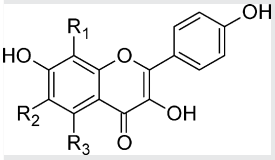
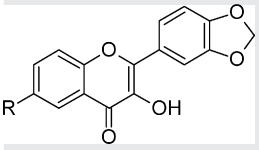
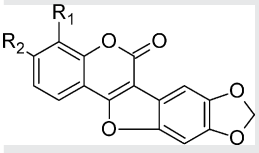
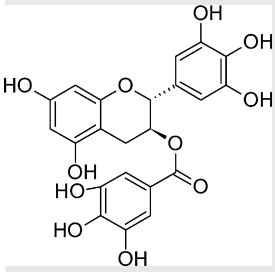
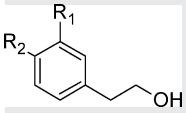
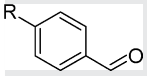
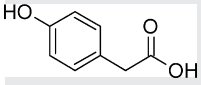
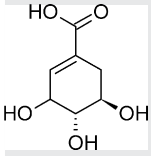
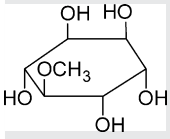
General structure	Compound type, names and substituents
	Xanthone Mangiferin[a]: R_1 = *C*-β-D-glucosyl; R_2 = H (1a,2a,3,4) Isomangiferin[a]: R_1 = H; R_2 = *C*-β-D-glucosyl (1a,2b,3,4)
	Flavanone Hesperidin[a,c]: R_1 = *O*-rutinosyl, R_2 = OH, R_3 = OCH_3, R_4 = OH (1a,2a,3,4) Hesperetin[a]: R_1 = R_2 = OH, R_3 = OCH_3, R_4 = OH (1a) Eriocitrin[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = R_4 = OH (1b,2,4) Eriodictyol[a]: R_1 = R_2 = R_3 = R_4 = OH (1a) Narirutin[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH, R_4 = H (1b,2a,3,4) Naringenin[a]: R_1 = R_2 = R_3 = OH, R_4 = H (1a) Prunin[b]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH, R_4 = H (1a) Naringenin-5-*O*-rutinoside[b]: R_1 = R_3 = OH, R_2 = *O*-rutinosyl, R_4 = H (1a) Eriodictyol-5-*O*-glucoside[b]: R_1 = R_3 = R_4 = OH, R_2 = *O*-β-D-glucosyl (1a) Eriodictyol-7-*O*-glucoside[b]: R_1 = *O*-β-D-glucosyl, R_2 = R_3 = R_4 = OH (1a)
	Flavone Luteolin[a,c]: R_1 = R_2 = R_3 = OH (1a,2a,3,4) Diosmetin[b]: R_1 = R_2 = OH, R_3 = OCH_3 (1a) 5-Deoxyluteolin[c]: R_1 = R_3 = OH, R_2 = H (2a) Scolymoside[c]: R_1 = *O*-rutinosyl, R_2 = R_3 = OH (2a)
	Isoflavone Formononetin[a]: R_1 = OH, R_2 = R_3 = R_4 = H, R_5 = OCH_3 (1a) Formononetin diglucoside[b]: R_1 = *O*-α-apiofuranosyl-(1‴ → 6″)-β-D-glucopyranosyl, R_2 = R_3 = R_4 = H, R_5 = OCH_3 (1a) Afrormosin[a]: R_1 = OH, R_3 = R_4 = H, R_2 = R_5 = OCH_3 (1a) Calycosin[a]: R_1 = R_4 = OH, R_2 = R_3 = H, R_5 = OCH_3 (1a) Wistin[b]: R_1 = *O*-β-D-glucosyl, R_3 = R_4 = H, R_2 = R_5 = OCH_3 (1a) Orobol[c]: R_1 = R_3 = R_4 = R_5 = OH, R_2 = H (2a)
	Methylinedioxyisoflavone derivative Pseudobaptigenin[a]: R = H (1a) Fujikinetin[a]: R = OCH_3 (1a)
	Flavonol Kaempferol-5-*O*-glucoside[b]: R_1 = R_2 = H, R_3 = *O*-β-D-glucosyl (1a) Kaempferol-6-*C*-glucoside[b,c]: R_1 = H, R_2 = *C*-β-D-glucosyl, R_3 = OH (1a) Kaempferol-8-*C*-glucoside[b]: R_1 = *C*-β-D-glucosyl, R_2 = H, R_3 = OH (1a) Kaempferol-8-*C*-glucoside[b]: R_1 = *C*-α-D-glucosyl, R_2 = H, R_3 = OH (1a)
	Methylinedioxyflavonol derivative 3′,4′-Methylenedioxyflavonol diglucoside[b]: R = *O*-α-apiofuranosyl-(1‴ → 6″)-β-D-glucopyranosyl (1a)
	Coumestan Medicagol[a]: R_1 = H, R_2 = OH (1a) Flemichapparin[a]: R_1 = H, R_2 = OCH_3 (1a) Sophoracoumestan B[a]: R_1 = OCH_3, R_2 = OH (1a)

Table 7 (*Continued*)

General structure	Compound type, names and substituents
	Flavan-3-ol (−)-epigallocatechin gallate[c] (2a)
	Phenylethanol derivative Tyrosol[b]: R_1 = H, R_2 = OH (1a) 3-Methoxy-tyrosol[b]: R_1 = OCH_3, R_2 = OH (1a) 4-Glucosyltyrosol[c]: R_1 = H, R_2 = *O*-β-D-glucosyl (2) Phenylethanol diglucoside[b]: R_1 = *O*-α-apiofuranosyl-(1″ → 6′)-β-D-glucopyranosyl, R_2 = H (1a)
	Benzaldehyde derivative Benzaldehyde diglucoside[b]: R = *O*-α-apiofuranosyl-(1″ → 2′)-β-D-glucopyranosyl (1a)
	Phenolic carboxylic acid *p*-Coumaric acid[a]: (1a)
	Organic acid (±)-Shikimic acid[c] (2a)
	Inositol (+)-Pinitol[a,c] (1a,2a)

(1a) identified in *Cyclopia intermedia* using NMR; (1b) identified in *Cyclopia intermedia* using LC–MS (Joubert et al., 2008b); (2a) identified in *Cyclopia subternata* using NMR; (2b) identified in *Cyclopia subternata* using LC–MS (Joubert et al., 2008b); (3) identified in *Cyclopia genistoides* using LC–MS (Joubert et al., 2008b); (4) identified in *Cyclopia sessiliflora* using LC–MS (Joubert et al., 2008b).

[a] Ferreira et al. (1998).
[b] Kamara et al. (2003).
[c] Kamara et al. (2004).

inating from other areas, Joubert et al. (2008b) showed that the TP content of *Cyclopia genistoides* was the least affected by fermentation (77% retention). Both *Cyclopia intermedia* and *Cyclopia subternata* retained 54%, while *Cyclopia sessiliflora* retained 58% of the TP content. Lower TP content after fermentation is attributed to the decrease in xanthone and flavonoid content with fermentation (Joubert et al., 2008b). The variations between samples of the same species, but harvested during different years and originating from different plantations, areas, age of the bush, etc., suggest that these factors could affect polyphenolic composition and thus susceptibility to oxidative degradation.

A preliminary investigation of the effect of harvest time on Overberg and West Coast types of *Cyclopia genistoides*, over a period of 15 weeks, starting at the end of March until mid-July, showed that the mangiferin content decreased slightly, but significantly during this period (5.92–5.21%). No significant change in isomangiferin and hesperidin contents was found (Joubert et al., 2003).

A cup of honeybush contains 0.59 μg/ml fluoride and 20.5 μg/ml Ca (Touyz and Smit, 1982). No information on the *Cyclopia* species that was analysed is available.

The volatile fraction of unfermented *Cyclopia genistoides* contains a large number of saturated and unsaturated alcohols, aldehydes and methyl ketones, while terpenoids comprise the major aroma fraction of the fermented plant material (Le Roux et al., 2008). The major constituent in unfermented plant material is 6-methyl-5-hepten-2-one, comprising 54% of the total ion

Table 8
Phenolic content of hot water extract of fermented *Cyclopia* species[a]

Compounds	*Cyclopia intermedia*	*Cyclopia subternata*	*Cyclopia genistoides*	*Cyclopia sessiliflora*
Mangiferin	0.23 ± 0.05	0.08 ± 0.02	4.29 ± 1.20	0.19 ± 0.04
Isomangiferin	nd	nd	0.94 ± 0.15	nd
Eriocitrin	nd	0.26 ± 0.07	nd	0.13 ± 0.04
Narirutin	0.02 ± 0.00	0.04 ± 0.01	0.20 ± 0.06	nd
Hesperidin	0.45 ± 0.08	0.27 ± 0.08	0.47 ± 0.01	0.45 ± 0.03
Hesperetin	0.06 ± 0.03	nd	nd	nd
Luteolin	nd	<0.01	0.01	nd
Total polyphenols[b]	16.26 ± 1.39	17.49 ± 1.54	22.01 ± 0.92	17.10 ± 1.44

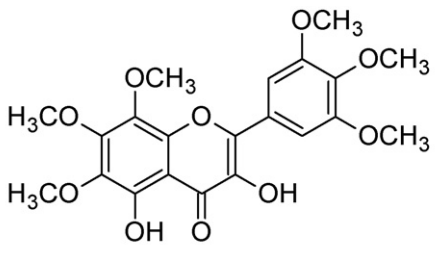
[a] Joubert et al. (2008b); values as percentage of dried extract; nd: not detected.
[b] Results expressed as gallic acid equivalents/100 g dried extract determined with Folin-Ciocalteu reagent.

chromatogram (TIC). Other compounds in relatively high concentrations (>0.9–11%) are linalool, limonene, hexenal, α-terpineol, 3,5-octadien-2-one, geranyl acetone, β-cyclocitral, dihydroactinidiolide, geraniol and trans-furanoid linalool oxide (in order of decreasing quantity). Linalool is the major constituent (36%) present in the aroma of fermented *Cyclopia genistoides*. Present in quantities of larger than 1% of the TIC are α-terpineol, 6-methyl-5-hepten-2-one, geraniol, nerol, limonene, *trans*-furanoid linalool oxide, hexenal and *cis*-furanoid linalool oxide (in order of decreasing quantity).

The composition of *Athrixia* spp. has not been extensively studied. Bohlmann and Zdero (1977) and Bohlmann et al. (1982) identified several diterpenes related to kaurene, triterpenes and thymol derivatives from root extracts of eight *Athrixia* spp. including *Athrixia phylicoides*. Aerial parts of *Athrixia pinifolia* yielded three diterpenes related to kaurene, while those of *Athrixia phylicoides* yielded germacren D, linoleic acid and *p*-hydroxyphenylpropan-3-ol-coumarate (Bohlmann and Zdero, 1977). Recently, a new flavonol derivative, 5-hydroxy-3′,4′,5′,6,7,8-hexamethoxyflavon-3-ol (Fig. 2), was isolated and identified from *Athrixia phylicoides* leaves (Mashimbye et al., 2006). Phenolic acids identified against authentic standards by HPLC with diode-array detection and LC–MS, included protocatechuic, *p*-coumaric, caffeic and chlorogenic acids, while several compounds with molecular ions corresponding to dicaffeoylquinic acids were also tentatively identified (De Beer and Joubert, 2007).

7. Biological properties

Most of the research on the biological properties of SA herbal teas has been done on rooibos. Fermented rooibos has been used almost exclusively in studies. Recent entry of unfermented rooibos on the market led to its inclusion in some studies. Most of the studies have been carried out either in South Africa, Japan or the Slovak Republic. In comparison the latter groups mostly used very dilute extracts in animal feeding experiments, corresponding to Japanese recommended concentration for human use (Table 2). This is also important when extrapolating data from animals to humans. Investigation of honeybush is very limited and partial to *Cyclopia intermedia*, mainly because it forms the bulk of exports. Very few studies investigated *Athrixia phylicoides*, but it has the distinction that it is the only indigenous South African tea that to date has been subjected to a sub-chronic toxicicity study.



5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavonol

Fig. 2. Unique new flavonol isolated from *Athrixia phylicoides* leaves.

7.1. Antioxidant properties

Antioxidant activity of teas and plant extracts is of great importance due to the ability of antioxidants to scavenge free radicals whereby cells in the human body could be protected against oxidative damage.

7.1.1. Extracts

Several studies were carried out to assess the antioxidant ability of rooibos extracts in a number of *in vitro* systems. Fermented rooibos was shown to scavenge the physiologically relevant reactive oxygen species, superoxide radical anion ($O_2^{\bullet -}$) (Yoshikawa et al., 1990; Standley et al., 2001; Joubert et al., 2004) and hydroxyl radical ($^{\bullet}OH$) (Yoshikawa et al., 1990; Lee and Jang, 2004; Joubert et al., 2005). Ito et al. (1991) and Yoo et al. (2008), respectively, demonstrated a protective effect for rooibos against oxidative stress caused by H_2O_2 in mouse L5178Y cells and Chinese hamster lung fibroblast V79-4 cells. Treatment with rooibos also induced the antioxidant enzymes, superoxide dismutase and catalase in V79-4 cells (Yoo et al., 2008). Protection against lipid peroxidation was demonstrated in several test systems including linoleic acid emulsions (Hitomi et al., 1999; Joubert et al., 2005), rabbit erythrocyte membranes (Hitomi et al., 1999), rat liver homogenates (Hitomi et al., 1999) and rat liver microsomes (Joubert et al., 2008b). Akaike et al. (1995) showed rooibos to scavenge alkyl peroxyl radicals formed during lipid peroxidation. Both a hot water and a 75% ethanol extract of rooibos inhibited peroxyl radical-induced DNA strand scission in a dose dependent manner, with the water extract being less effective (Lee and Jang, 2004). The effectiveness of the extracts was similar to the major antioxidant of green tea (*Camellia sinensis*), epigallocatechin gallate (EGCG). Using the deoxyribose assay, the rooibos extracts were shown to be ineffective in scavenging $^{\bullet}OH$, but able to protect DNA against attack from $^{\bullet}OH$ generated from H_2O_2, due to their ability to scavenge H_2O_2. Joubert et al. (2005) demonstrated $^{\bullet}OH$ scavenging for the hot water extract of unfermented rooibos at high concentrations in the reaction mixture (>89 μM dihydrochalcone content).

Comparison of unfermented and fermented rooibos hot water extracts demonstrated that their antioxidant activity, as measured in the $O_2^{\bullet -}$, DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical), ABTS$^{\bullet +}$ (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation) and FRAP (ferric reducing antioxidant potential)

Table 9
Comparison of the antioxidant activity of hot water extract of unfermented and fermented *Aspalathus linearis* (updated from Joubert and Schulz, 2006)

Assay	Parameter	Unfermented rooibos	Fermented rooibos	Reference(s)
FRAP	Total antioxidant activity[a]	1.98	1.45	Joubert et al. (2008b)
$ABTS^{\bullet+}$ scavenging	Total antioxidant activity[b]	2.37	1.72	Joubert et al. (2008b)
$DPPH^{\bullet}$ scavenging	% Scavenging[c]	86.6	83.4	Von Gadow et al. (1997a)
	% Scavenging[d]	87.3	83.0	Joubert et al. (2004)
	EC_{50}[e]	2.33	3.62	Adapted from Standley et al. (2001)
	EC_{50}[e]	3.24	3.87	Joubert et al. (2004)
	Rate of scavenging[f]	8.3×10^{-4}	7.35×10^{-4}	Winterton (1999)
$O_2^{\bullet-}$ scavenging	IC_{50}[g]	44.4	60.5	Adapted from Standley et al. (2001)
	IC_{50}[g]	69.4	78.3	Joubert et al. (2004)
Linoleic acid emulsion oxidation	% Inhibition (CD)[h]	28.6	28.0	Joubert et al. (2005)
β-Carotene-linoleic acid oxidation	AAC[i]	557	607	Von Gadow et al. (1997a)
Sunflower oil-in-water emulsion oxidation	% Inhibition (peroxides)[j]	90.0	80.9	Winterton (1999)
	Induction time (PV)[k]	35	31	
	% Inhibition (CD)[l]	58.1	54.5	
Methyl linoleate micelles oxidation	% Inhibition (TBARS)[m]	22.8	30.3	Winterton (1999)
Rat liver microsomal peroxidation	% Inhibition (TBARS)[n]	51.91	41.07	Joubert et al. (2008b)

Abbreviations: ABTS: 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant potential.
[a] μmol Trolox equivalents/mg of dried extract.
[b] μmol Trolox equivalents/mg of dried extract.
[c] Scavenging (%) of $DPPH^{\bullet}$ (6×10^{-5} M) after 70 min.
[d] Scavenging (%) of $DPPH^{\bullet}$ (3.04×10^{-5} M) after 20 min.
[e] Effective concentration of dried extract (μg/ml) in reaction mixture required to scavenge 50% of $DPPH^{\bullet}$ (3.04×10^{-5} M).
[f] $DPPH^{\bullet}$ rate of scavenging (s^{-1}), calculated during unsteady state conditions (time 0–3 min), expressed as the change in the absorbance at 515 nm over time.
[g] Concentration of dried extract (μg) per ml reaction mixture required to inhibit 50% of NBT reduction.
[h] Inhibition (%) of conjugated diene (CD) formation after 21 h incubation at 40 °C.
[i] Antioxidant activity coefficient (AAC) measured as inhibition of β-carotene discolouration.
[j] Inhibition of peroxides after 35 days incubation at 30 °C.
[k] Time required for oxidation to reach a peroxide value (PV) of 10 meq/kg oil with incubation at 30 °C.
[l] Inhibition of CD formation after 31 days incubation at 30 °C.
[m] Inhibition of the formation of thiobarbituric reactive substances (TBARS) after 16 h incubation at 37 °C.
[n] Inhibition of the formation of TBARS during Fe^{2+}-induced microsomal lipid peroxidation after 1 h incubation at 37 °C.

assays (Table 9), decreases as a result of fermentation. The effect is attributed to a decrease in TP content with fermentation. Standley et al. (2001), investigating the influence of processing stages (i.e. before and after fermentation, as well as after sun-drying, sieving and steam pasteurisation) demonstrated that fermentation had the greatest effect on $O_2^{\bullet-}$ and $DPPH^{\bullet}$ scavenging abilities, resulting in decreased radical scavenging ability. Sun-dried fermented rooibos was slightly more effective in scavenging $O_2^{\bullet-}$ than the artificially dried plant material, but the opposite was true for $DPPH^{\bullet}$. Steam pasteurisation slightly improved the $DPPH^{\bullet}$ scavenging ability of the tea extract, but it had no effect on its $O_2^{\bullet-}$ scavenging ability. The only processing stage that affected pro-oxidant activity was the fermentation step, leading to decreased pro-oxidant activity, which was attributed to the substantial lowering in dihydrochalcone content with fermentation (Joubert et al., 2005).

The protective effect of unfermented and fermented rooibos against lipid peroxidation was not consistent, with some assays showing the hot water extract prepared from fermented rooibos to be more effective than or not significantly different from the unfermented rooibos extract (Table 9). A high concentration of

Table 10
Comparison of antioxidant activity of hot water extracts of fermented and unfermented *Cyclopia* species

	$ABTS^{\bullet+}$ scavenging	$DPPH^{\bullet}$ scavenging	FRAP	$O_2^{\bullet-}$ scavenging	Linoleic acid emulsion oxidation	Rat liver microsomal peroxidation
Parameter[a]	TAA	EC_{50}	TAA	IC_{50}	% inhibition (CD)	% inhibition (TBARS)
Cyclopia intermedia						
Unfermented	2.11	5.1	1.70	170.5	58.5	36.4
Fermented	0.96	12.3	0.75	238.2	66.2	23.6
Cyclopia subternata						
Unfermented	2.03	6.5	1.66	96.7	88.2[b]	36.2
Fermented	0.94	15.2	0.78	151.8		24.7
Cyclopia genistoides						
Unfermented	1.99	5.8	1.47	109.9	91.3[b]	31.2
Fermented	1.44	10.0	0.97	132.4		33.1
Cyclopia sessiliflora						
Unfermented	1.97	3.8	1.63	60.8	84.8[b]	42.0
Fermented	0.95	5.5	0.78	97.0		24.2
Cyclopia maculata						
Unfermented	nd	5.5	nd	158.8	78.7[b]	nd
Fermented	nd	13.2	nd	249.3		nd
References	Joubert et al. (2008a)	Hubbe (2000)	Joubert et al. (2008a)	Hubbe and Joubert (2000)	Hubbe (2000)	Joubert et al. (2008a)

[a] Parameters and abbreviations as defined in Table 9. nd: not determined.
[b] Values for unfermented and fermented teas were pooled as no significant ($p < 0.05$) differences were observed between unfermented and fermented teas.

polyphenols, especially highly potent flavonoids, such as present in the extracts of unfermented rooibos, could result in pro-oxidant activity, countering the antioxidant effect (Joubert et al., 2005). Using the so-called Fenton reaction with stimulation of deoxyribose degradation as endpoint, it was found that the dihydrochalcone and flavonoid contents, but not TP content of rooibos extracts correlated with pro-oxidant activity (Joubert et al., 2005.).

Selective extraction with ethyl acetate, resulting in enhanced flavonoid concentration, increased both the DPPH$^{\bullet}$ and $O_2^{\bullet -}$ scavenging abilities of the unfermented and fermented rooibos dried extract (Joubert et al., 2004), as well as the pro-oxidant activity of the extracts, as measured in the deoxyribose assay (Joubert et al., 2005). Tannin, isolated from unfermented rooibos, displayed antioxidant activity in both radical scavenging assays (Joubert et al., 2004) and was able to inhibit lipid peroxidation in a linoleic acid emulsion (Joubert et al., 2005). In all assays its activity was lower than that of the hot water extracts of unfermented and fermented rooibos. Its pro-oxidant activity was similar to that of fermented rooibos hot water extract.

Exposure of the extract to an additional period of heating, simulating the traditional practice of keeping the brew hot on the stove for extended periods, increased the induction period for a fixed ratio of hot water dried extract of fermented rooibos to lard as measured with the Rancimat method, suggesting beneficial compositional changes with prolonged heat exposure (Von Gadow et al., 1997b). However, no effect for extraction time or additional heat exposure could be demonstrated with the β-carotene bleaching method (Von Gadow et al., 1997b). The TP and total flavonoid content of the hot water soluble solids of the extract, prepared in a flow-through batch system, did not change after the initial 5–8 min of extraction (Joubert, 1990c). Investigation of the extraction kinetics of aspalathin in water at 80 °C showed that ca. 50% is extracted within 5 min, reaching a plateau between 60 and 90 min (Jaganyi and Wheeler, 2003). It is not known what the effect of extraction time and heating would be on the extract antioxidant activity in cellular or *in vivo* model systems.

Two studies investigated the relative order of antioxidant efficiency of the hot water dried extracts of unfermented and fermented rooibos and the *Camellia sinensis* teas, green, oolong and black (Von Gadow et al., 1997a; Joubert et al., 2008b). Five to seven samples of each were used, to take into account compositional variation within a type. Irrespective of the test system and the set of samples used, green tea (*Camellia sinensis*) dried extracts were the most effective. In other studies using either one sample or a small number of samples, the antioxidant activity of rooibos was compared with a range of herbal teas. Rooibos (fermented) is less effective than mate (*Ilex paraguarensis* A. St.-Hill) (Piccinelli et al., 2004; Ivanova et al., 2005) and burrito (*Wendita calysina*), a Paraguayan tea that is used as alternative to green tea. Rooibos (fermented) is, however, substantially more effective as a scavenger of $^{\bullet}$OH and $O_2^{\bullet -}$ radicals than roselle, also known as red tea (*Hibiscus sabdariffa* L.) (Steenkamp et al., 2004).

The relative antioxidant activity of hot water extracts of *Cyclopia* species is summarised in Table 10. Unfermented extracts, containing a higher concentration of TP, irrespective of species, had consistently higher antioxidant activity in the $O_2^{\bullet -}$, DPPH$^{\bullet}$, ABTS$^{\bullet +}$ and FRAP assays than their fermented counterparts. Fermentation reduced the ability of the dried extracts of *Cyclopia intermedia*, *Cyclopia subternata* and *Cyclopia sessiliflora* to inhibit Fe^{2+}-induced microsomal lipid peroxidation. However, fermentation had no significant effect on the inhibitory ability of *Cyclopia genistoides*, possibly due to pro-oxidant activity as a result of high mangiferin content (Joubert et al., 2008b). Another set of unfermented and fermented samples of *Cyclopia maculata* (ex Genadendal), *Cyclopia sessiliflora*, *Cyclopia subternata* and *Cyclopia genistoides* did not differ in their ability to inhibit linoleic acid peroxidation (Hubbe, 2000). Fermentation, however, decreased the protective effect of *Cyclopia intermedia* in the same assay. Selective extraction of unfermented and fermented *Cyclopia intermedia* with methanol substantially decreased its $O_2^{\bullet -}$ scavenging ability, compared to the water extracts (Hubbe and Joubert, 2000). Separating the plant material of fermented *Cyclopia maculata* (ex Du Toit's Kloof) into fractions ≤2 mm and >2 mm (containing more woody material and coarse leaves), Hubbe (2000) showed that the extracts prepared from the fraction ≤2 mm were more effective scavengers of DPPH$^{\bullet}$ and $O_2^{\bullet -}$, due to the dried extract having a higher TP content. However, by using longer extraction times for the fraction >2 mm, i.e. 15 min and 30 min, their scavenging ability of $O_2^{\bullet -}$ and DPPH$^{\bullet}$, respectively, could be increased to equal that of a 5-min extraction of the ≤2 mm fraction.

An aspect that should be highlighted is that the relevant antioxidant activity of the species depends not only on the assay used, but also on the sample set, due to intra species compositional variation. Therefore, at this stage no species can be singled out as having the highest antioxidant activity.

Honeybush, in general, compared to *Camellia sinensis* and rooibos teas, has the lowest antioxidant activity in *in vitro* antioxidant assays (Table 11). However, with the sample set used by Joubert et al. (2008b), some of the *Cyclopia* species had higher or equal activity than the other teas, except green tea and unfermented rooibos. For instance, unfermented *Cyclopia intermedia* had a higher ferric reducing ability than fermented rooibos, oolong or black teas, while unfermented *Cyclopia subternata* and *Cyclopia sessiliflora* were comparable to black tea, and unfermented *Cyclopia genistoides* was comparable to fermented rooibos, black and oolong teas. The protective effect of *Cyclopia sessiliflora* against microsomal lipid peroxidation was comparable to that of fermented rooibos (Joubert et al., 2008b). Steenkamp et al. (2004) demonstrated higher $^{\bullet}$OH and $O_2^{\bullet -}$ scavenging ability for *Cyclopia intermedia* than for roselle. *Cyclopia intermedia* was less effective than mate in the ABTS$^{\bullet +}$ scavenging assay (Ivanova et al., 2005).

Pro-oxidant activity observed for rooibos and honeybush extracts implies that a $^{\bullet}$OH could be generated in the presence of iron which has implications *in vivo*, especially under disease conditions when iron is mobilised. As a critical balance exists between antioxidant and pro-oxidant activity, the final concentration of the specific polyphenol will determine the biological outcome. This, however, will depend on the absorption and biotransformation of the polyphenolic constituents.

Very few studies have investigated the antioxidant activity of *Athrixia* spp. Antioxidant activity of *Athrixia phylicoides* and *Athrixia elata* hot water infusions, hot water decoctions, cold water extracts and cold ethanol extracts were determined using the ABTS$^{\bullet +}$ scavenging assay (McGaw et al., 2007). *Athrixia phylicoides* extract showed similar antioxidant activity to rooibos tea, while being less active than black tea (*Camellia sinensis*). Ethanol extracts gave lower activity than the water-based extracts, while *Athrixia elata* extracts were less active than *Athrixia phylicoides* extracts. Trends for antioxidant activity corresponded to trends observed for TP content. *Athrixia phylicoides* water extracts were also shown to have good antioxidant activity in the ABTS$^{\bullet +}$ scavenging, DPPH$^{\bullet}$ scavenging, FRAP, linoleic acid emulsion peroxidation and microsomal lipid peroxidation assays (De Beer and Joubert, 2007). Activity was generally higher than that of commercial honeybush tea extracts, but lower than that of commercial rooibos tea extracts, confirming its potential as an antioxidant-rich extract.

Table 11
Relative order of antioxidant activity of hot water extracts of *Aspalathus linearis*, *Cyclopia* species and *Camellia sinensis* in different assays

Assay	Parameter[a]	*Aspalathus linearis*[b] and *Camellia sinensis* teas	*Aspalathus linearis* and *Cyclopia* species[c]	References
ABTS$^{\bullet+}$ scavenging	TEAC	Green > RNS > black		Piccinelli et al. (2004)
	TEAC	Green > RNS	RNS > C.int (F)	Ivanova et al. (2005)
	TEAC	White ≈ green > black ≫ RNS		Almajano et al. (2008)
	TAA	Green > black > RU ≈ oolong > RF	RU > all *Cyclopia* spp. RF > all fermented *Cyclopia* spp. C.gen (U) ≈ C.sess (U) ≈ C.sub (U) ≈ C.int (U) > C.gen (F) > C.int (F) ≈ C.sess (F) ≈ C.sub (F)	Joubert et al. (2008b)
	VCEAC	Green > black > RNS		Yoo et al. (2008)
DPPH$^{\bullet}$ scavenging	%Scavenging	Green > RU > RF > RSF > black > oolong		Von Gadow et al. (1997a)
	EC_{50}	Green > black > RNS	RF ⋙ honeybush[d]	Du Toit et al. (2001)
	%Scavenging	Green ≈ black > RNS		Yoo et al. (2008)
FRAP	TAA	Green > RU > black > RF ≈ oolong	RU > all *Cyclopia* spp; RF ≈ C.gen (U) < other unfermented *Cyclopia* spp. > all fermented *Cyclopia* spp.; C.int (U) ≥ C.sub (U) ≈ C.sess (U) > C.gen (U) > C.gen (F) > C.sub (F) = C.sess (F) ≈ C.int (F)	Joubert et al. (2008b)
$O_2^{\bullet-}$	% Scavenging		RNS > C.int (F)	Steenkamp et al. (2004)
OH$^{\bullet}$	% Scavenging		RNS > C.int (F)	Steenkamp et al. (2004)
Chemiluminescence	TEAC	Green > black > RNS		Piccinelli et al. (2004)
Sunflower oil-in-water emulsion	PV	Green ≈ RNS > black > white		Almajano et al. (2008)
Linoleic acid oxidation	% Inhibition	Green < RNS	RNS > honeybush[d]	Lindsey et al. (2002)
β-Carotene bleaching method (coupled linoleic acid oxidation)	AAC	Green > black > oolong > RF > RU > RSF		Von Gadow et al., 1997a
Microsomal lipid peroxidation	% Inhibition	Green > RU ≈ black ≈ oolong > RF	Only C.sess (U) ≈ RF; all unfermented and fermented *Cyclopia* spp. < RF C.sess (U) > C.int (U) ≈ C.sub (U) ≈ C.gen (F) ≈ C.gen (U) > C.sub (F) ≈ C.int (F) ≈ C.sess (F)	Joubert et al. (2008b)

[a] Parameters and abbreviations defined as in Table 9, except for EC_{50}: μg dried extract/ml; PV: peroxide value; TEAC: Trolox equivalent antioxidant capacity; VCEAC: vitamin C equivalent antioxidant capacity (mg VCE/100 g plant material).
[b] RU: unfermented rooibos; RF: fermented rooibos; RSF: semi-fermented rooibos; RNS: fermentation state not specified.
[c] C.gen: *Cyclopia genistoides*; C.sess: *Cyclopia sessiliflora*; C.sub: *Cyclopia subternata*; C.int: *Cyclopia intermedia*; C.mac: *Cyclopia maculata*; U: unfermented; F: fermented.
[d] *Cyclopia* species not specified.

7.1.2. Compounds

The importance of aspalathin, as novel and major rooibos flavonoid, as well as potential quality marker of unfermented rooibos, required comparison of its antioxidant activity with other rooibos flavonoids in different assays. The relative order of activity was determined, using the DPPH$^{\bullet}$ and $O_2^{\bullet-}$ assays (Von Gadow et al., 1997c; Joubert et al., 2004). Of the different compounds tested, aspalathin consistently outperformed most of the rooibos flavonoids. Aspalathin was slightly less effective than quercetin in scavenging DPPH$^{\bullet}$ (Von Gadow et al., 1997c; Joubert et al., 2004), while their $O_2^{\bullet-}$ (Joubert et al., 2004) and ABTS$^{\bullet+}$ (Snijman, 2007) scavenging abilities were comparable. Quercetin is a potent antioxidant in various systems (Silva et al., 2002), as well as a pro-oxidant (Laughton et al., 1989). The very low quercetin content in rooibos, however, does not mean that it would not make a meaningful contribution to an *in vivo* antioxidant effect, since its glycosides are present in substantial quantities (Bramati et al., 2002), and deglycosylation occurs in the intestines (Manach et al., 2004). The DPPH$^{\bullet}$ scavenging ability of isoquercitrin and rutin is, respectively, comparable to and lower than that of aspalathin (Joubert et al., 2004). Both compounds have similar $O_2^{\bullet-}$ scavenging ability. The flavone analogues of aspalathin, orientin and *iso*-orientin, as well as other flavonoids tested, i.e., luteolin, (+)-catechin and chrysoeriol are less effective than aspalathin in scavenging DPPH$^{\bullet}$ and $O_2^{\bullet-}$. Procyanindin B3, the flavanol dimer, displayed higher activity than aspalathin in both assays (Joubert et al., 2004). The absence of the 3-OH on the B ring of nothofagin resulted in a lower ABTS$^{\bullet+}$ scavenging activity than aspalathin, although it remains one of the rooibos flavonoids with high activity (Snijman, 2007). Its flavone analogues, vitexin (Von Gadow et al., 1997c; Joubert et al., 2004) and isovitexin (Snijman, 2007), however, are very poor radical scavengers. Except for caffeic acid, with a DPPH$^{\bullet}$ scavenging ability comparable to quercetin, and slightly better than aspalathin, other rooibos phenolic acids, i.e. ferulic acid, *p*-coumaric acid, syringic acid, vanillic acid, protocatechuic acid and *p*-hydroxybenzoic acid, have lower activities (Von Gadow et al., 1997c).

In lipid peroxidation systems, other factors, e.g. phase distribution and molecular configuration, are important in determining antioxidant activity and as such influence the relative order of activity of compounds. Rooibos flavonoids serve as a good example: aspalathin was substantially less effective than luteolin and quercetin in the β-carotene bleaching method, but only slightly more effective than vitexin, a poor radical scavenger (Von Gadow et al., 1997c). The antioxidant activity coefficient (AAC) value of vitexin was 88% of that of aspalathin. Isoquercitrin and rutin displayed, respectively, 63 and 23% of the AAC value of aspalathin. In the Rancimat test, protection offered by the compounds against lipid peroxidation, in increasing order of induction time was aspalathin < rutin < isoquercetin ⋘ quercetin (Von Gadow et al., 1997c). In the Fe^{2+}-induced microsomal lipid peroxidation system, aspalathin was much more effective than nothofagin, suggesting that iron chelation by aspalathin played an important role in this test system (Snijman et al., 2003).

The honeybush xanthone, mangiferin, also occurs in other plants, most notably in *Mangiferin indica* L. Bark from this tree is used to produce an antioxidant extract high in mangiferin content (Núñez-Sellés, 2005). Several papers have dealt with its antioxi-

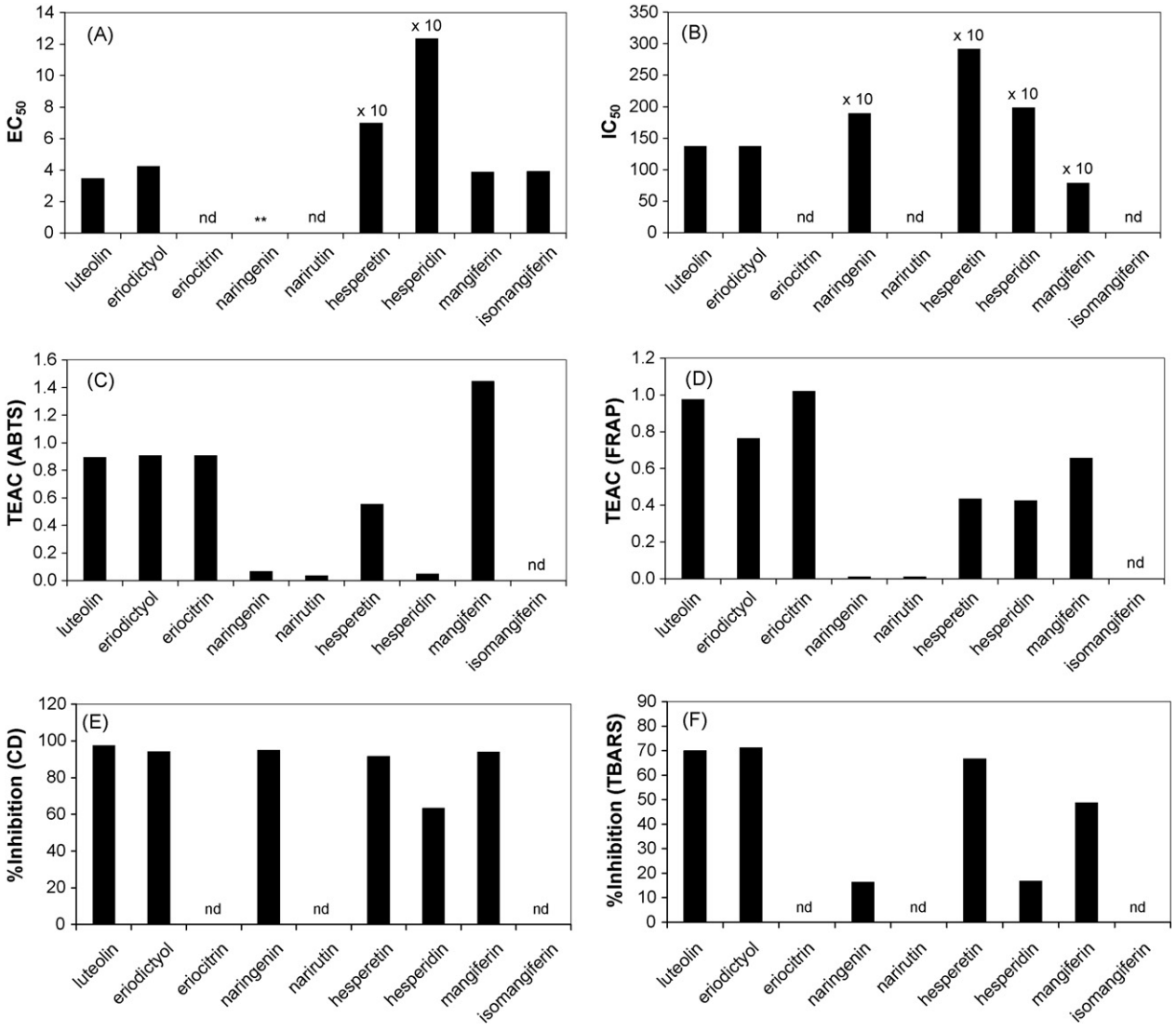


Fig. 3. Relative antioxidant activity of *Cyclopia* polyphenols—data for: (A and E) from Hubbe (2000), (B) from Hubbe and Joubert (2000) and (C, D and F) from Joubert et al., 2008b. (A) DPPH• scavenging activity [effective concentration of compound (μM) in reaction mixture required to scavenge 50% of DPPH• (3.04×10^{-5} M)]; (B) $O^{\bullet -}$ scavenging activity [concentration of compound (μM) per ml reaction mixture required to inhibit 50% of NBT reduction]; (C) $ABTS^{\bullet +}$ scavenging activity [Trolox equivalent antioxidant capacity as mM Trolox equivalents required to give the same activity as 1 mM of compound]; (D) ferric reducing antioxidant potential [Trolox equivalent antioxidant capacity as mM Trolox equivalents required to give the same activity as 1 mM of compound]; (E) linoleic acid lipid peroxidation assay [inhibition (%) of conjugated diene (CD) formation after 21 h incubation at 40 °C]; (F) rat liver microsomal peroxidation [inhibition of the formation of TBARS during Fe^{2+}-induced microsomal lipid peroxidation after 1 h incubation at 37 °C]; nd: not determined; (**) no activity at tested concentrations; 10×: values should be multiplied by 10 for actual values.

dant activity and other compounds that are found in honeybush, such as hesperidin. In the context of this review, the comparative antioxidant activity of mangiferin and *Cyclopia* polyphenols (Hubbe, 2000; Hubbe and Joubert, 2000; Joubert et al., 2008b) are of interest (Fig. 3). Mangiferin was shown to be one of the most active antioxidants of the *Cyclopia* polyphenols tested. Due to its high concentration in the extracts, its contribution to the antioxidant activity would be substantial. Isomangiferin was slightly less effective than mangiferin in the DPPH• scavenging assay. Luteolin, present in very small quantities, was the most active of the compounds tested in the DPPH• and $O_2^{\bullet -}$ scavenging assays, but it was less effective than mangiferin in scavenging $ABTS^{\bullet +}$. Eriodictyol gave comparable $O_2^{\bullet -}$ scavenging activity to luteolin. It also gave comparable activity to its rutinoside eriocitrin in the $ABTS^{\bullet +}$ scavenging assay. Hesperidin, the other major polyphenol of *Cyclopia* species, demonstrated very weak scavenging ability for DPPH•, $ABTS^{\bullet +}$ and $O_2^{\bullet -}$. The flavanone naringenin, the isoflavone formononetin and the coumestans flemichapparin and medicagol were ineffective scavengers of DPPH•. Very weak activity for naringenin and its rutinoside narirutin was demonstrated in the $ABTS^{\bullet +}$ scavenging and FRAP assays. Formononetin and the coumestans were ineffective scavengers of $O_2^{\bullet -}$. These compounds lack the structural features essential (Rice-Evans et al., 1996) for effective radical scavenging.

In lipid systems, luteolin and eriodictyol were the most effective, while naringenin was ineffective as inhibitor of conjugated diene formation in a linoleic acid emulsion, and poorly inhibited Fe^{2+}-induced microsomal lipid peroxidation. Mangiferin and isomangiferin were slightly less effective than luteolin in inhibiting linoleic acid peroxidation. Notably, hesperidin displayed poor inhibition of lipid peroxidation in both test systems.

Conversion of the glycosides *in vivo* to their corresponding aglycones will generally improve their antioxidant activity. However, the antioxidant activity of norathyriol is weaker than that

of butylated hydroxytoluene (BHT) in the DPPH• scavenging assay (Rukachaisirikul et al., 2006), while its glycosides, mangiferin and isomangiferin, are more effective in scavenging DPPH• than BHT (Hubbe, 2000). *In vitro* testing of *Cyclopia* extracts cannot reflect the full measure of their potential as antioxidants *in vivo*. Bioavailability of these compounds from complex matrices such as the extracts needs to be investigated.

7.2. Antimutagenic/anticancer properties

Interest in the possible role of flavonoids in chemoprevention is due to their modulation of carcinogen action in the cell. Flavonoid–cell interactions affect the cell's oxidative status and levels of xenobiotic metabolising enzymes, whereby the binding of reactive carcinogenic metabolites to macromolecules, such as protein and DNA, is inhibited and/or reduced. The modulation of cell signalling pathways, affecting cell growth parameters related to cell proliferation and apoptosis, are key events determining cell survival.

7.2.1. Mutagen specific responses

Initial studies on the antimutagenic properties of hot water extracts of rooibos and honeybush (*Cyclopia intermedia*) were conducted in the *Salmonella* mutagenicity assay against strains TA 98, TA 100 and TA 102 (Marnewick et al., 2000). These strains screen for different types of carcinogens including those causing frame shift and base-pair substitution mutations, as well as DNA changes associated with oxidative damage, in the presence and absence of metabolic activation. The extracts of rooibos and honeybush exhibited the highest protection against carcinogens requiring metabolic activation. Inhibition was more effective when the extracts were present in close proximity to the liver homogenate fraction implying interference with carcinogen activation via interaction with the phase I drug metabolising enzyme, cytochrome P450. Weaker protection was noticed when carcinogen activation and the *Salmonella* bacteria with the extracts were in different compartments (double layer technique), suggesting that the tea constituents also directly interact with the reactive mutagenic metabolite. The weaker direct protection was further confirmed, as a far less effective protection was noticed against direct acting mutagens implying that tea interference, presumably by the polyphenolic constituents, with the drug metabolising enzymes is the key event in explaining the antimutagenic properties of the herbal teas. The comutagenic response of some of the extracts in the presence of direct acting mutagens could be ascribed to specific extract–mutagen interactions.

7.2.2. Modulation of carcinogen metabolising enzymes

Apart from the interference with carcinogen activation, other protective parameters were also investigated in a subsequent study by monitoring the effect of rooibos and honeybush (*Cyclopia intermedia*) hot water extracts on the activity of drug metabolising enzymes in the liver of rats (Marnewick et al., 2003). A 10-week exposure treatment with extracts of fermented and unfermented rooibos and honeybush as sole source of drinking fluid significantly increased the activity of the cytosolic glutathione *S*-tranferase α. The unfermented teas significantly increased the activity of microsomal UDP-glucuronosyl transferase. The oxidative status of the liver was significantly altered by increasing the GSH:GSSG ratio (ratio of reduced glutathione to oxidised glutathione) due to increased GSH and decreased GSSG levels. The modulation of these parameters would increase the removal of reactive mutagenic metabolites *in vivo* via phase II reactions, thereby effectively protecting against carcinogen tissue interactions. An *ex vivo* study showed that cytosolic liver fractions of rats that consumed hot water extracts of fermented and unfermented rooibos and honeybush for 10 weeks protected against aflatoxin B_1 (AFB_1)-induced mutagenesis (Marnewick et al., 2004). The cytosolic liver fraction of rats drinking the unfermented teas protected against 2-acetylaminofluorene (2-AAF)-induced mutagenesis, while marginal effects were noticed with the fermented teas. Microsomal preparations prepared from the rats fed rooibos and unfermented honeybush extracts also reduced the mutagenic response to AFB_1. In contrast, microsomes prepared from rats fed unfermented honeybush tea (*Cyclopia intermedia*) enhanced mutagenesis of 2-AAF. Although the level of cytochrome P450 was not altered, it was suggested that isoforms of the enzyme involved in the metabolic activation of these carcinogens, are selectively decreased or increased by the tea constituents, presumably the polyphenols.

Studies in genetically modified Chinese hamster lung fibroblasts, expressing cytochrome P450 1A2 (CYP1A2) and the sulphotransferase 1C1 (SULT1C1), were utilised to monitor the antigenotoxic effects of rooibos against 2-AAF and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) with the Comet assay (Edenharder et al., 2002). The genotoxicity of PhIP was moderately inhibited by rooibos, while a much stronger inhibitory effect was noticed against 2-AAF. The antimutagenic effects are thought to be via the inhibition of CYP1A2 and SULT1C1 as the tea suppressed the genotoxicity of benzo[*a*]pyrene-7,8-dihydrodiol and 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, substrates of the respective enzymes.

Differences in the modulation of the type II substrate binding to cytochrome P450 by unfermented rooibos and *Cyclopia subternata*, *Cyclopia intermedia*, *Cyclopia genistoides* and *Cyclopia sessiliflora* were demonstrated spectroscopically (Van der Merwe, 2004). Of the tea flavonoids, luteolin, hesperetin, chrysoeriol and eriodictyol, showed significant alterations in the type II binding, while the glycosides, aspalathin, mangiferin and hesperidin, only exhibited weak inhibitory effects. These differences were related to the polarity of the flavonoids and/or the interaction with other binding sites of the enzyme. The effect of rooibos constituents on the P450 dependent adrenal enzyme system related to glucocorticoid biosynthesis was also monitored using progesterone as substrate (Richfield, 2008). Fermented rooibos exhibited the highest inhibitory effects of the mixed type of progesterone binding, specifically to CYP21. The more hydrophobic rooibos extracts showed stronger competitive inhibition than the more hydrophilic extracts, which exhibited a non-competitive inhibition. In contrast, hydrophobic column fractions also showed strong non-competitive inhibition of progesterone binding.

7.2.3. Comparative antimutagenic properties of extracts

Studies were conducted to compare the effect of tea processing on the antimutagenic potency of rooibos and honeybush. Tea fermentation significantly reduced the antimutagenic potency of rooibos tea, while subsequent steps, including sun-drying, sieving and pasteurisation, only exhibited marginal reductive effects (Standley et al., 2001). However, a subsequent study (Van der Merwe et al., 2006) showed that fermented rooibos exhibited a higher antimutagenic effect than its unfermented counterpart. In both studies, random sampling of plant material was carried out, so that the discrepancy could be due to phenolic variation of the plant material, and not only due to processing. The role of processing became evident as *Cyclopia intermedia*, *Cyclopia subternata* and *Cyclopia sessiliflora* fermentation significantly reduced the protective effect against 2-AAF- and AFB_1-induced mutagenesis (Van der Merwe et al., 2006). In this study, fermented and unfermented plant material used for extract preparation came

from the same plant, so that variation in polyphenolic composition and antimutagenic properties could directly be linked to processing. For comparative purposes *Camellia sinensis* teas were included in the study. In general, black tea exhibited the highest protective properties followed by oolong ≈ green ≈ fermented rooibos > unfermented rooibos ≈ unfermented honeybush > fermented honeybush. Of the honeybush teas, unfermented *Cyclopia intermedia* and unfermented *Cyclopia subternata* exhibited similar protection to that of fermented rooibos against AFB_1-induced mutagenesis. Against 2-AAF, fermented rooibos exhibited a similar protection to that of unfermented *Cyclopia intermedia* and *Cyclopia sessiliflora*. Fermented *Cyclopia intermedia* and *Cyclopia genistoides* exhibited the lowest protection against 2-AAF-induced mutagenesis, while fermented *Cyclopia intermedia* had the lowest protection against AFB_1-induced mutagenesis. The type of mutagen, therefore, will affect the specific order of potency, due to the specific metabolic pathway involved in the generation of the reactive mutagenic metabolite. Of interest was the enhancement of the mutagenic response of 2-AAF by the extracts of unfermented *Cyclopia genistoides*. In this regard the stabilisation of cytochrome P450 during the *in vitro* mutagenicity assays, due to the antioxidant potency of the herbal tea, appears to increase the formation of the active mutagenic metabolite derived from 2-AAF (Van der Merwe, 2004; Joubert et al., 2006b).

7.2.4. Comparative antimutagenic properties of polyphenols

Antimutagenesis studies on the rooibos polyphenolic constituents showed that aspalatin, nothofagin and their flavone derivatives exhibit moderate antimutagenic properties when compared to epigallocatechin gallate (EGCG) (Snijman et al., 2007). Luteolin and chrysoeriol, minor constituents of rooibos, exhibit similar antimutagenic properties to that of EGCG, implying that interaction between the different major flavonoids and/or unknown constituents are responsible for the antimutagenic properties of fermented and unfermented rooibos. This can be deduced from the type of dose response effects and flavonoid–mutagen interactions obtained (Snijman et al., 2007). Dose response effects were mutagen specific and included typical to atypical, threshold and biphasic responses. In addition to the antimutagenic effects, different flavonoid–mutagen interactions occurred that ranged from comutagenic, promutagenic and mutagenic responses. Using 2-AAF as mutagen, chrysoeriol exhibited a comutagenic response at high and low concentrations in the presence of metabolic activation, which, as described for the water extract of unfermented *Cyclopia genistoides*, could be related to the stabilisation of cytochrome P450. Depending on the dose, quercetin and isoquercitrin exhibited antimutagenic, promutagenic and mutagenic effects in the presence and absence of metabolic activation. Since the concentrations of these flavones are extremely low, they are not likely to affect the antimutagenic properties of rooibos, although synergistic interactions between different polyphenolic constituents are possible. It is not known at present whether synergistic interactions occur or to what extent it may affect the antimutagenic activity of tea extracts.

The honeybush polyphenols, hesperidin, eriocitrin and mangiferin, exhibited similar, but moderate antimutagenic properties compared to EGCG, when using AFB_1 as mutagen. Hesperetin and eriodictyol had similar protective activity to EGCG against AFB_1-induced mutagenesis. A much weaker protection was noted against 2-AAF-induced mutagenesis with eriodictyol, hesperidin and narirutin exhibiting a comutagenic response. The relative contribution of these polyphenols could vary depending on the plant material, impacting on the quantitative variation in antimutagenic activity (Van der Merwe et al., 2006).

7.2.5. Anticarcinogenic properties in vivo

The inhibition of tumour promotion in mouse skin by unfermented and fermented rooibos and honeybush (*Cyclopia intermedia*) was conducted using ICR mice with dimethylbenzantrachene as initiator and 2-O-tetradecanoyl-phorbol 13-acetate (TPA) as cancer promoter (Marnewick et al., 2005). Topical application of a non polar extract of the different teas prior to TPA significantly reduced the number and size of the skin tumours in the order unfermented honeybush > fermented honeybush > fermented rooibos > unfermented rooibos. As TPA-induced tumour promotion is associated with chronic inflammation, the role of hesperidin and mangiferin, as major antioxidants of honeybush, will be of interest. Hesperidin (Guardia et al., 2003; Rotelli et al., 2003), its aglycone hesperetin (Rotelli et al., 2003) and mangiferin (Leiro et al., 2003) possess anti-inflammatory activity.

A study related to cancer promotion in mouse skin showed that the expression of COX-2 in ICR mouse skin by TPA was significantly reduced by a methanol extract of rooibos (Na et al., 2004). A similar response was noticed in MCF10A human breast cancer cells, with rooibos inhibiting the binding of the transcription factor NF-kB, known to regulate COX-2 expression. The bioactive rooibos constituents responsible are not known at present. The major flavonoid in unfermented rooibos, aspalathin, is poorly absorbed (0.07–0.08%) in the different layers of human skin in Franz diffusion cells (Huang et al., 2008). In contrast, absorption of aspalathin in intestinal cell monolayers was 80 and 100% when using pure aspalathin and a flavonoid enriched extract, respectively. It is of interest that aspalathin is more readily absorbed when present in an enriched extract as compared to the pure form.

The cancer modulating properties of rooibos and honeybush teas were also monitored in liver and oesophagus cancer models in male Fischer 344 rats. Unfermented rooibos and honeybush (*Cyclopia intermedia*) protected against fumonisin B_1 (FB_1)-induced cancer promotion in diethylnitrosamine-initiated rat liver as the number and size of early pre-neoplastic lesions, staining positively for the placental form of gamma glutamyl transferase (PGST) were reduced (Marnewick, 2004). Specific FB_1-tissue-polyphenolic interactions, altering different oxidative parameters in the liver, are likely to be involved. As fermentation tends to reduce the protective effect of rooibos and honeybush teas, differences in their major polyphenolic components may explain the varying effects of the different teas on the oxidative parameters, hepatotoxic effects and the inhibitory potential against cancer promotion. Sissing (2008), using a methylbenzylnitrosamine (MBN)-induced rat oesophageal cancer model in male Fischer 344 rats, demonstrated cancer modulating properties for unfermented honeybush (*Cyclopia intermedia*) and rooibos. Both teas significantly reduced the number and size of papillomas while marginal effects was also noticed with fermented honeybush. The MBN-treated rats receiving unfermented rooibos and fermented and unfermented honeybush also failed to develop larger papillomas (>10 and >20 mm^3). The reduction in the proliferative capacity of the papillomas and/or pre-neoplastic lesions in the liver could be important in developing chemopreventive strategies against oesophageal and liver cancer utilising the rooibos and honeybush teas.

Cell viability studies conducted in human oesophageal cancer cells (WHCO5) showed that unfermented and fermented rooibos was more effective in reducing the ATP production than the honeybush counterparts (Sissing, 2008). The findings were similar for inhibition of cell proliferation. Differences in the polyphenols were suggested to explain the selective cytotoxic and anti-proliferative effects of the herbal teas in WHC05 cells.

7.3. Hepatoprotective effects

Based on the known antioxidant properties of rooibos several studies were conducted on its hepatoprotective properties utilising different hepatotoxicants. Chronic treatment of male Wistar rats with CCl_4 twice weekly for 10 weeks resulted in steatosis and cirrhosis (Uličná et al., 2003). Changes in the blood clinical chemical parameters included an increase in the aminotransferases, alkaline phosphatase, total bilirubin and albumin, while the concentrations of glucose, triacylglycerols, cholesterol and creatinine decreased. A water extract of rooibos as their sole drinking fluid and a once a day gavage treatment significantly reduced the CCl_4-induced plasma markers for liver damage, whilst protecting against hepatic steatosis and fibrosis. The induction of lipid peroxidation caused by the reactive cytochrome P450 metabolites of CCl_4 was completely prevented by the water extract treatment. In a follow-up experiment, utilising similar experimental conditions, rooibos increased the antioxidant status of the CCl_4-damaged livers, by increasing the α-tocopherol and reduced coenzyme Q_9 levels to values comparable to healthy animals (Kucharská et al., 2004). The treatment with rooibos also inhibited the formation of malondialdehyde (MDA), an indicator of lipid peroxidation, which is associated with CCl_4-induced hepatic injury. It was suggested that rooibos could be applied as a hepatoprotector in the diet of patients with liver disease. A study in streptozotocin-induced diabetic rats receiving rooibos showed that the water and alkaline rooibos extracts did not affect the markers for the diabetic status, but decreased the plasma markers related to hepatotoxic effects (Uličná et al., 2006). Advanced glycation end products (AGEs) and oxidation products (MDA), regarded as end products of oxidative stress, were reduced in the plasma, liver, kidney and lens by rooibos. The protection against oxidative stress was ascribed to the presence of antioxidant compounds present in rooibos. An *in vitro* study also showed that rooibos water extract decreased the formation of AGEs in a dose dependent manner after incubation of glucose with serum albumin. The suppression activity was associated with the $O_2^{\bullet -}$ scavenging activity of rooibos during the initial stages of the Maillard reaction (Kinae et al., 1994).

A study in vitamin E deficient Wistar rats showed that supplementation of their feed with freeze-dried hot water extract of rooibos tends to decrease the level of lipid peroxidation in several organs, including liver, small intestine and stomach but changes were not significant (Hitomi et al., 2004). A similar trend was noticed when the susceptibility to lipid peroxidation induced by 2,2′-azobis(2-amidinopropane) were monitored. No significant changes were noticed when monitoring the glutathione and lipid hyperoxide levels or the activity of glutathione peroxidase.

7.4. Phyto-oestrogenic properties

Phyto-oestrogens are non-steroidal, polyphenolic secondary metabolites from plants with a structural and functional similarity to the endogenous human hormone, oestrogen (Dixon, 2004). Their potential to modulate oestrogen signalling has been the focus of recent scientific interest and phyto-oestrogens are considered as alternatives for the treatment of menopausal symptoms and steroid hormone dependent cancers such as breast, prostate, endometrial and colon cancer (Adlercreutz and Mazur, 1997; Kurzer and Xu, 1997; Cassidy et al., 2000; Cornwell et al., 2004; Usui, 2006; Whelan et al., 2006; Amin and Buratovich, 2007). Despite reported beneficial effects of phyto-oestrogens results have, however, not always been favourable or reproducible, which may be attributed to, amongst others, the fact that a wide variety botanicals in different doses have been used and that standardisation of formulations are not currently required (Cornwell et al., 2004; De Lima Toccafondo Vieira et al., 2008). Rooibos and honeybush tea have also been shown to possess oestrogenic activity, while oestrogenic activity of bush tea (*Athrixia phylicoides*) has not been evaluated.

Only one publication (Shimamura et al., 2006) reported investigation of the phyto-oestrogenic activity of rooibos methanol extracts and compounds present in the extracts. Shimamura et al. (2006) isolated 25 compounds from the methanol extracts of rooibos and found that nothofagin had the highest oestrogenic activity, while three other compounds, isovitexin, luteolin-7-glucoside, and hemiphlorin, displayed moderate oestrogenicity. Luteolin, quercetin, eriodictyol and some of their glycosides, were also present, but did not display appreciable oestrogenic activity despite the fact that binding of these molecules to the oestrogen receptor (ER) has been reported (Markaverich and Gregory, 1993; Oh and Chung, 2004; Verhoog et al., 2007a). The ELISA kit used by Shimamura et al. (2006) to test for oestrogenic activity uses an antibody that specifically recognises estrone (E_1), 17β-estradiol (E_2) and estriol (E_3). Whether this would necessarily translate into ER binding, by definition required of a phyto-oestrogen (Kurzer and Xu, 1997), is debatable.

Initial screening for phyto-oestrogenicity in *Cyclopia* focused on ER binding, the first step in oestrogenic signalling. Specifically, the ability of water and methanol extracts of four *Cyclopia* species, *Cyclopia genistoides*, *Cyclopia subternata*, *Cyclopia sessiliflora*, and *Cyclopia intermedia*, to displace tritiated E_2 (^{3}H-E_2) from human ERα and ERβ (Verhoog et al., 2007a). The water extracts showed no significant displacement from ERα, while water extracts from several different batches of *Cyclopia genistoides* and *Cyclopia subternata* plant material showed displacement from ERβ (Table 12). Generally, water extracts from unfermented material showed higher ER binding. Screening of methanol extracts was only conducted with unfermented *Cyclopia* species. Two extracts prepared from *Cyclopia genistoides* and *Cyclopia subternata* showed significant binding to ERα, while three extracts (two from different batches of samples of *Cyclopia genistoides*, and one from *Cyclopia subternata*) showed significant binding to ERβ. One *Cyclopia genistoides* and one *Cyclopia subternata* extract showed strong and significant binding to both ER isoforms. In addition, several polyphenols, with potential oestrogenic activity, previously found to be present in *Cyclopia* (Table 7), was evaluated for binding to the ER isoforms. Luteolin, naringenin, and formononetin, like genistein and E_2, bound to both ER isoforms, while eriodictyol, eriocitrin, and narirutin bound only to ERβ. Hesperidin and mangiferin, the most abundant polyphenols in *Cyclopia*, did not bind to either ER isoform (Verhoog et al., 2007a). HPLC analysis of the specific *Cyclopia* extracts investigated indicated the presence of eriocitrin and narirutin, but not luteolin, formononetin, naringenin, or eriodictyol. The lack of correlation between the amount of eriocitrin and narirutin present in the extracts and the extent of displacement of ^{3}H-E_2 from either ERα or ERβ, coupled to the relatively low levels of these polyphenols in the most active fractions, suggest that these polyphenols alone could not account for the observed binding to the ER isoforms.

A detailed investigation of the methanol extracts of three *Cyclopia genistoides* harvestings (P104, P105 and P122) and four polyphenolic compounds present in *Cyclopia*, that had either been shown to bind to both ER isoforms (luteolin, formononetin, and naringenin) or was present at very high concentration (mangiferin), was conducted (Verhoog et al., 2007b). Whole cell binding studies to human ERα and ERβ transiently transfected into COS-1 cells confirmed that all the polyphenols tested, except mangiferin, bound to both ER subtypes. The polyphenols, with the exception of formononetin, bound preferentially to ERβ, with affinities significantly lower than that of E_2. The dried methanol extracts (DME), although all from the same species, displayed large variation in

Table 12
Relative order of oestrogenic activity of extracts from fermented and unfermented *Cyclopia* spp.

Assay	System	Parameter	Relative order of extracts	Reference(s)
Whole cell ER-binding	COS-1 cells transiently transfected with hERα	% Displacement of 1 nM ^{3}H-E_2	DAE (U): no significant displacement DAE (F): no significant displacement DME (U): C.gen = C.sub ≫ C. sess = C.int	Verhoog et al. (2007a)
	COS-1 cells transiently transfected with hERβ	% Displacement of 1nM ^{3}H-E_2	DAE (U): C.gen = C.sub ≫ C. sess = C.int DAE (F): C.gen ≫ C.sub = C. sess = C.int DME (U): C.gen > C.sub > C. sess = C.int	Verhoog et al. (2007a)
	MCF-7-BUS cells with endogenous ER	IC_{50}	DME (U): C.sub > C.gen	Mfenyana (2008)
Promoter-reporter assay	T47D-KBluc cells that contain stably transfected $(ERE)_3$-containing promoter and luciferase reporter	EC_{50}	DME (U): C.gen > C.sub	Mfenyana (2008)
Alkaline phosphatase assay	Ishikawa Var-1 cells	EC_{50}	DME (U): C.gen = C.sub C.sub (U): S-Met > S-Eth > S-Hlf = N-Eth = N-Met = N-Hlf = COT > N-EAc > S-EAc ≫ S-Wat = N-Wat	Mfenyana (2008)
E-screen	Proliferation in MCF-7-BUS cells measured using MTT assay	EC_{50}	DME (U): C.sub > C.gen C.sub (U): S-Met > S-Eth > S-Hlf = N-Eth = N-Met = N-Hlf > N-EAc > S-EAc > COT ≫ S-Wat = N-Wat	Mfenyana (2008)

Abbreviations: C.gen: *Cyclopia genistoides*; C.int: *Cyclopia intermedia*; COT: "cup-of-tea" extracts; C.sess: *Cyclopia sessiliflora*; C.sub: *Cyclopia subternata*; DAE: dried aqueous extracts; DME: dried methanol extracts; EAc: ethyl acetate extracts; Eth: ethanol extracts; F: fermented; Met: methanol extracts; Hlf: 50% methanol/water extracts; N: non-sequential extraction; S: sequential extraction; U: unfermented; Wat: water extracts.

binding with the DME of only one harvesting, P104, binding to both ER isoforms (Table 13). The extracts from harvesting P104, unlike the polyphenols tested, did not bind preferentially to ERβ, but rather displayed a higher affinity for ERα. The P104 extract, however, also displayed an affinity significantly lower than that of E_2. Despite preferential binding to ERα by the P104 extract and no ER binding by the other DMEs, all DMEs transactived the vitelligin-oestrogen response element promoter reporter construct in COS-1 cells via human ERβ, but not via ERα. The polyphenols tested, except mangiferin, could transactivate via both ERs but generally more potently, with the exception of luteolin, via ERβ.

All the polyphenols tested and the DMEs from P104 and P105 caused proliferation of MCF-7 breast cancer cells, albeit to a significantly lesser extent than E_2. In addition, DMEs from the same two harvestings, but not the polyphenols or E_2, induced proliferation of MDA-MB-231 breast cancer cells. Co-treatment with ICI 182,780, an ER antagonist, established that proliferation of MCF-7 cells was fully ER dependent, while proliferation of MDA-MB-231 cells was only partially mediated through ER. This suggests that the DMEs of *Cyclopia* may mediate some of their proliferative effects in MDA-MB-231 cells via an ER-independent mechanism. All the polyphenols, except mangiferin, and all the DMEs, including that of P122 that on its own caused no proliferation, reduced

Table 13
Comparison of the oestrogenic activity of dried methanol extracts (DME) from unfermented *Cyclopia genistoides*

Assay	System	Parameter	Range of values	Reference(s)
Whole cell ER-binding	COS-1 cells transiently transfected with hERα	% Displacement of 1 nM ^{3}H-E_2	9–74%	Verhoog et al. (2007a)
		IC_{50}	NB: 2.1×10^{-4} mg/ml	Verhoog et al. (2007b)
	COS-1 cells transiently transfected with hERβ	% Displacement of 1 nM ^{3}H-E_2	NB: 70%	Verhoog et al. (2007a)
		IC_{50}	NB: 1.3×10^{-1} mg/ml	Verhoog et al. (2007b)
	MCF-7-BUS cells with endogenous ER	IC_{50}	NB: 1.67×10^{-4} mg/ml	Mfenyana (2008)
Promoter-reporter assay	COS-1 cells transiently transfected with hERα and $(vitERE)_2$-containing promoter and luciferase reporter	EC_{50}	NA	Verhoog et al. (2007b)
	COS-1 cells transiently transfected with hERβ and $(vitERE)_2$-containing promoter and luciferase reporter	EC_{50}	2.48×10^{-6} to 9.20×10^{-5} mg/ml	Verhoog et al. (2007b)
	T47D-KBluc cells that contain stably transfected $(ERE)_3$-containing promoter and luciferase reporter	EC_{50}	NA: 5.13×10^{-4} mg/ml	Mfenyana (2008)
Alkaline phosphatase assay	Ishikawa Var-1 cells	EC_{50}	NA: 1.89×10^{-3} mg/ml	Mfenyana (2008)
Breast cancer proliferation	Proliferation in MCF-7-BUS cells measured using MTT assay	EC_{50}	NA: 1.98×10^{-6} mg/ml	Verhoog et al. (2007b)
			NA: 6.48×10^{-3} mg/ml	Mfenyana (2008)
	Proliferation in MDA-MB-231 cells measured using MTT assay	EC_{50}	NA: 1.39×10^{-10} mg/ml	Verhoog et al. (2007b)
SHBG-binding	Binding in DCC stripped human pregnancy plasma	% Displacement of 20 nM ^{3}H-E_2	29.3–37.0%	Verhoog et al. (2007b)

Abbreviations: NA: no activity; NB: non-binder; other abbreviations as defined in Table 12.

E_2-induced (1 nM) proliferation of MCF-7 cells, suggesting antagonistic action within a pre-menopausal milieu (Verhoog et al., 2007b). Furthermore, all polyphenols, including mangiferin, and all DMEs displaced E_2 from sex hormone binding globulin (SHBG). HPLC and LC–MS analysis of the DMEs indicated that of the active polyphenols, only luteolin was present at low concentrations. Interestingly, the HPLC peaks eluting at retention times corresponding to narirutin and eriodictyol were shown by LC–MS to be of unknown flavanone glycosides, suggesting that the identification of these polyphenols in the first *Cyclopia* study was probably erroneous (Verhoog et al., 2007a). In addition, three other unknown peaks, two with UV–vis spectra similar to those of flavanone glycosides, were observed. As the luteolin levels in the DMEs tested were too low to explain the oestrogenic activity observed, it was suggested that these unknown polyphenols may possibly confer oestrogenic activity on the *Cyclopia* DMEs. *Cyclopia* extracts display the two attributes of phyto-oestrogens, weak oestrogenicity and preference for ERβ, which have been proposed to contribute to beneficial health benefits (Magee and Rowland, 2004). Ligands acting preferentially through ERβ are sought, as it has been suggested that ERβ may act as a negative regulator of ERα in antagonising its proliferative action in cancer cells (Magee and Rowland, 2004; Mak et al., 2006). Further evidence supporting the action of *Cyclopia* extracts via ERβ comes from their ability to antagonise E_2-induced cell proliferation (Verhoog et al., 2007b).

Four other *Cyclopia* harvestings, three from *Cyclopia genistoides* (M7-9) and one from *Cyclopia subternata* (M6), were evaluated for oestrogenicity so that an extract with enhanced oestrogenicity could be prepared for benchmarking against commercial extracts (Mfenyana, 2008). The two harvestings with the highest oestrogenicity, M6 and M7, were sequentially and non-sequentially extracted with five solvents of increasing polarity (ethyl acetate, ethanol, methanol, 50% methanol-distilled water and distilled water). The sequentially extracted M6 methanol extract had the highest potency and the sequentially extracted M6 ethyl acetate extract had the highest efficacy in the alkaline phosphatase and MCF-7 proliferation (E-screen) assays, respectively (Table 12). HPLC and LC–MS data indicated a positive correlation between luteolin content and efficacy, while the content of an unidentified flavanone correlated best with potency. In addition, an extract mimicking the making of a cup of tea, the popular way of ingesting *Cyclopia*, also displayed oestrogenic activity, suggesting that this mode of consumption could result in ingestion of phyto-oestrogens. Benchmarking against four commercial phyto-oestrogen nutraceuticals, Phytopause Forte®, a soy isoflavone extract, Promensil®, a red clover isoflavone extract, Remifemin®, a black cohosh extract, and Femolene Ultra®, a combination of extracts from several plants including soy, black cohosh, Mexican wild yam, and maidenhair tree, indicated that the *Cylopia* DMEs from sequential methanol and ethyl acetate extraction of *Cyclopia subternata* had, respectively, comparable efficacy and potency to the commercial nutraceutical products, suggesting their potential to compete in the market place. However, although *Cyclopia* extracts from specific harvestings look promising, no blanket claim for the oestrogenicity of honeybush tea can be made as great intra and inter *Cyclopia* species variability of oestrogenic potency has been observed (Table 13).

7.5. Miscellaneous

7.5.1. Antispasmodic effects

The potential therapeutic use of rooibos in K^+ channel activation and smooth muscle relaxation for the treatment of asthma has been recognised, based on studies in the jejunum (Gilani et al., 2006). A concentrated rooibos extract, containing approximately 120 and 199 mg quercetin equivalents/g extract of total polyphenols and flavonoids, respectively, exhibited bronchodilatory, antispasmodic and blood pressure lowering effects in experimental animals including rabbits, guinea-pigs and rats (Khan and Gilani, 2006). The extract was shown to contain a K_{ATP} channel dependent bronchodilatory substance as it relaxes the K^+-induced contractions in guinea-pig trachea and rabbit aorta, while the mean arterial blood pressure in rats was significantly reduced by rooibos in a dose dependent manner. Studies utilising pure rooibos flavonoids and phenolic acids showed that chrysoeriol, orientin and vitexin exhibited K_{ATP} channel opening activity in the jejunum (Gilani et al., 2006). Chrysoeriol was found to be 25 times and 40 times more potent in the trachea when compared to the jejunum and aorta, respectively (Khan and Gilani, 2006). Orientin was inactive in the trachea, while vitexin exhibited similar activities in the jejunum and trachea. The medicinal use of rooibos against hyperactive gastrointestinal and respiratory problems needs to be further explored.

7.5.2. Immune system modulation

Kunishiro et al. (2001) showed that rooibos water extract stimulated antibody production in murine splenocytes when using anti-ovalbumin (OVA) and sheep red blood cells, while it lacked any effects in lipopolysaccharide-stimulated (LPS) splenic B-cells. In splenocytes, rooibos increased the generation of interleukin (IL)-2 primed with OVA and CD3, while it suppressed the production of IL-4 in OVA primed cells. Oral administration of a rooibos water extract to Wistar rats significantly restored the OVA-induced antibody production after cyclosporin A treatment. Rooibos also stimulated IL-2 generation in murine splenocytes *in vivo,* although there was no difference between OVA-stimulated and control mice. Ichiyama et al. (2007) showed than an aqueous fraction, obtained after column fractionation of a hot water extract of rooibos, increased the immunoglobulin M (IgM) production in anti-OVA-stimulated murine splenocytes, which was associated with the production of IL-10. A similar effect was noticed *in vivo* with a rooibos water extract increasing the anti-OVA IgM level in sera of BALB/c mice (Ichiyama et al., 2007). These effects are known to suppress the formation of IL-2 and IFN-γ by T cells, thereby stimulating proliferation and differentiation of B cells and the antibody production process. These immunological effects were not associated with the presence of the major rooibos flavonoids such as aspalathin, orientin and rutin, but more with the presence of oligosaccharides and polysaccharides.

7.5.3. Protection against cell transformation, cell proliferation and clastogenic effects

A water extract of rooibos significantly reduced X-ray-induced C3H10T1/2 cell transformation in a dose dependent manner when considering the morphological alterations related to oncogenic transformation (Komatsu et al., 1994). No effect was noticed with green tea (*Camellia sinensis*). A possible role of antioxidant components of rooibos was suggested to mediate the inhibitory effect. Anticlastogenic effects of rooibos were also demonstrated in CHO cells treated with benzo[*a*]pyrene and mitomycin C (MMC) (Sasaki et al., 1993; Shimoi et al., 1994). Rooibos decreased the induction of chromosomal aberrations in the presence of a liver homogenate fraction, implying interference with metabolic activation. Rooibos also suppressed chromosomal aberrations of MMC in the absence of metabolic activation. The role of antimutagenic flavonoids present in rooibos, acting as desmutagens or bio-antimutagens, was suggested. Rooibos water extract also inhibited the formation of micronuclei in mice, dosed orally 6 h prior to MMC or gamma ray treatment. A daily rooibos tea intake of 6–8 cups by a per-

son weighing 60 kg was calculated to simulate the mouse intake. In a subsequent experiment, fractionation of the rooibos tea extract showed that the flavonoid fraction, exhibiting antioxidant activity in the bone marrow and spleen, has the highest activity against the induction of micronucleated reticulocytes following gamma irradiation (Shimoi et al., 1996). Luteolin was characterised as an effective component in the rooibos extract, showing anticlastogenic and antioxidant activity against lipid peroxidation in the Fenton reaction.

Little information is available on the antiproliferative properties of rooibos. A study in embryonic chick skeletal muscle cells, including primary cells, fibroblasts and myoblasts, showed that rooibos inhibited cell proliferation in a dose dependent manner (Lamošová et al., 1997). The inhibitory effects were linked to the potent antioxidant activity of the rooibos water extract.

Athrixia phylicoides has not yet been investigated for anticancer/antimutagenic properties. A dichloromethane extract of the seeds and leaves of *Athrixia elata*, however, exhibited moderate growth inhibitory, cytostatic and cytotoxic effects against three cancer cell lines, namely TK10 (renal cancer), MCF7 (breast cancer) and UACC62 (melanoma) (Fouche et al., 2006).

7.5.4. Vasodilatory effect

Persson et al. (2006) investigated the effect of rooibos on the angiotensin-converting enzyme (ACE), which amongst others, catalyses conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, and production of the vasodilator, nitric oxide (NO), using cultured endothelial cells from human umbilical veins as model system. Incubation of the cells with rooibos for 10 min showed no significant inhibition of ACE, but 24 h incubation resulted in a significant dose dependent increase in NO production.

7.5.5. Antihemolytic effect

The protection against free radical-mediated damage of erythrocytes occurs via enzymatic and non-enzymatic systems in which dietary antioxidants may play an important contributing role. The antioxidant effect of rooibos tea was therefore investigated, utilising erythrocytes as a model system for oxidative damage of biomembranes (Simon et al., 2000). Rooibos, fed to Japanese quails either by supplementing their drinking fluid with rooibos extract or the feed with milled plant material for 180 days, did not change the fragility of erythrocytes either to peroxide- or hypotonia-induced hemolysis. Rooibos inhibited peroxide-induced hemolysis in the erythrocytes to the same extent as ascorbic acid. This effect is presumably related to the antioxidant capacity of the flavonoids in rooibos tea.

7.5.6. Anti-ageing properties

When comparing rats at 5-week- and 24-months-old, age related accumulation of lipid peroxides in the brain was closely associated with a decrease in magnetic resonance imaging due to lipid denaturation (Inanami et al., 1995). The high level of lipid peroxidation in aged rat brain was suggested to be the result of increased spontaneous free radical generation together with a reduction in defence mechanisms. Rooibos extract, fed after weaning, significantly decreased the accumulation of lipid peroxides in several regions of the brain in the 24-month-old rats, presumably due to the presence of flavonoids scavenging $O_2^{\bullet -}$ and $^{\bullet}OH$.

Since birds are regarded as a suitable model of ageing in biomedical research (Holmes and Austad, 1995), Japanese quails were used by Juráni et al. (2008) to study the effect of rooibos on their productive life. By substituting drinking water with rooibos extract or supplementing the diet with milled rooibos plant material, the decrease in egg production of aged Japanese quail hens was reduced, thereby prolonging their productive period. Long-term drinking of rooibos had no effect on their body weight. The positive effect was attributed to either antioxidant or phyto-oestrogenic activities of the tea.

7.5.7. Antimicrobial and antiviral effects

Hot water extracts and their ethyl acetate extracts, prepared from both fermented and unfermented rooibos, were shown to have a growth inhibitory effect against *Escherichia coli* in a dose dependent manner (Scheepers, 2001). The fermented rooibos water extract was more effective than the unfermented rooibos extract, giving, respectively, a 69 and 35% decrease in growth at 5 mg/ml. The ethyl acetate extracts, prepared to selectively enhance the monomeric flavonoid content, were not as effective as the water extracts. However, for the ethyl acetate extracts, growth inhibition was only slightly improved with increasing concentrations of fermented rooibos extract, while it remained constant for the unfermented rooibos extract. Growth profiles carried out after the cells were removed from the mediums containing the water extracts of unfermented and fermented rooibos, showed that *Escherichia coli* was able to resume growth, indicating that the rooibos extracts are bacteriostatic.

Scheepers (2001) also showed that the hot water extracts of unfermented and fermented rooibos inhibited the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans* and *Saccharomyces cerevisiae*. The extracts, especially those of fermented rooibos, stimulated growth of *Zygosaccharomyces rouxii*. The extracts were most effective against *Staphylococcus aureus*, with the fermented extract more effective than the unfermented extract. At a concentration of 2 mg/ml (equaling approximately the concentration in a cup of tea), 21 and 57% growth inhibition of *Staphylococcus aureus* after 12 h was observed for the extracts of unfermented and fermented rooibos, respectively.

Using zone inhibition on solid media, Almajano et al. (2008) demonstrated antimicrobial activity for rooibos water extract against the bacteria *Bacillus cereus* and *Micrococcus luteus* and the yeast *Candida albicans*. No inhibitory activity was shown in this test system against *Pseudomonas aeruginosa*, *Escherichia coli* and *Lactobacillus acidophilus*.

Coetzee et al. (2008) demonstrated that extracts of unfermented rooibos and honeybush (*Cyclopia subternata* and *Cyclopia genistoides*) at 10 mg/ml inhibited growth of *Escherichia coli* in liquid cultures by 60–85% after 6 h, but inhibition was 25–50% after 24 h, and decline further thereafter. In the same study the potential antifugal activity of unfermented rooibos and *Cyclopia subternata* extracts against the plant pathogen, *Botrytis cinerea* was investigated. When tested at 10 mg/ml both extracts stimulated biomass production by more than 3-fold after 24 h. At 100 mg/ml the rooibos and *Cyclopia subternata* extracts reduced spore germination of *Botrytis cinerea* by 33 and 17%.

An alkaline water extract of rooibos, prepared from plant material that was first subjected to hot water extraction, suppresses HIV-induced cytopathicity of HIV (HTLV-III) infected MT-4 cells *in vitro* (Nakano et al., 1997a, b). It had a strong anti-HIV activity (EC_{50} = 38.9 μg/ml) and low cytotoxicity (CC_{50} = 2400 μg/ml). The hot water extract was not effective. Fractionation of the alkaline extract showed that the active substances were mainly recovered in the 25–75% ethanol-precipitated fraction (EC_{50} = 18.3 μg/ml), containing an acidic polysaccharide composed of 26% reducing sugars (mainly glucose), 49% neutral sugars (mannose, galactose and xylose) and 15–25% uronic acid. At a concentration of 250 μg/ml, it almost completely blocked binding of HIV to MT-4 cells, which suggested its involvement in the initial step in the replication of HIV (Nakano et al., 1997b).

7.6. Human studies

7.6.1. Dermatological effects

A study was conducted in patients with viral (*herpes simplex*) infection, itching or disease related to reactive oxygen species (Shindo and Kato, 1991). Patients received a dilute infusion of rooibos at least once a week, which decreased the incidence of *herpes simplex* within 2–3 days. Patients with atopic dermatitis were successfully treated, resulting in a decrease in the itching sensation. Inflammatory disease with infiltration of polymorphic neuclocytes tends to subside after treatment with rooibos. A similar effect was noticed during steroid-induced dermatitis, which was also successfully treated with rooibos. Photosensitivity as a result of UV irradiation was also controlled and treatment with glucocorticoid could be reduced.

7.6.2. Anti-allergic effect

A study conducted in humans monitoring the effect of a type I skin response after a skin prick test using 16 common allergens showed no modulating effect by rooibos (Hesseling and Joubert, 1982). Volunteers consumed large quantities of rooibos (500 ml three times a day for 1 week). In addition, a rooibos poultice (500 g in 500 ml H_2O) was also applied to one arm 15 min before the skin prick test. Despite these treatments the size of the skin induration remained unchanged, which raises doubts concerning the therapeutic value of rooibos in allergic disease in the skin, lungs and nose. On the other hand variation in composition, i.e too low concentrations or absence of specific compounds, could be a contributing factor to the inability to demonstrate an anti-allergic effect.

7.6.3. Effect on antioxidant status of blood

The effect of an aspalathin-enriched extract of unfermented rooibos, containing 15% aspalathin, on the antioxidant status of the blood plasma of 20 study subjects after a washout period of 1 week was investigated by Sauter (2004). They received a twice daily oral dose of 250 mg extract in tablet form for 2 weeks. Their diet was restricted to ensure a low flavonoid intake during this period. No significant changes in blood parameters or antioxidant status of the plasma, as measured with the $ABTS^{\bullet+}$ and Cu^{2+}-induced low-density lipoprotein oxidation test systems, was demonstrated. However, the antioxidant status of the plasma as measured with the xanthine/xanthine oxidase test system showed a slight decrease after supplementation of the diet with the enriched rooibos extract.

An 8 weeks randomised placebo-controlled intervention study was conducted to monitor the effect of rooibos drinking on the antioxidant status of workers occupationally exposed to lead (Nikolova et al., 2007). Indices such as blood lead levels, antioxidant status, including superoxide dismutase, GSH and lipid peroxidation, were monitored in the red blood cells and plasma. Rooibos decreased the lipid peroxidation level, whilst increasing the GSH concentration. No effect on the lead levels was observed.

7.6.4. Effect on iron absorption

The effect of rooibos on iron absorption was investigated by comparing its effect on the uptake of 1 μCi ^{59}Fe-citrate/Fe-sulfate in the presence of ascorbic acid (Hesseling et al., 1979). The study subjects (10 males per group) either received 200 ml rooibos, black tea (*Camellia sinensis*) or water each containing 20 ml milk and 20 g sugar. The serum parameters, monitored after 14 days, including hemoglobin, ferritin, transferrin, serum iron and iron binding capacity, failed to indicate any effect for rooibos, while black tea significantly reduced iron uptake. A recent study in school children showed no adverse effects of rooibos or black tea on the iron status of school children (Breet et al., 2005). Children received a 200 ml serving twice a day, containing 40 ml milk and 8 g of sugar per serving, over a period of 16 weeks. A detailed 24-h recall questionnaire was completed to assess iron intakes. There were no significant changes in the iron status parameters, including the serum ferritin, transferrin, the total iron binding capacity and the transferrin saturation, between the different groups. However, the iron status tended to increase towards the end of the study, which was ascribed to antheimintic treatment of the children.

8. Safety and toxicity

The historical and modern use of rooibos and honeybush as a beverage for everyday consumption has led to a general assumption of its safety as no reports of toxicity, at the normal use as herbal tea, has been doumented. No toxicological studies have been done, however, a number of studies have addressed aspects of safety and toxicity of rooibos. Chronic consumption of aqueous extracts of unfermented and fermented rooibos and honeybush by rats over a period of 10 weeks did not cause any adverse effects in the liver and kidney, considering the serum biochemical data. The serum iron and cholesterol levels were also not significantly altered (Marnewick et al., 2003). Subsequent studies are in progress to further evaluated herbal tea/iron interactions under different physiological conditions, including different liver toxicity models involving iron.

Rooibos tea exhibited a mutagenic effect when considering sex coupled recessive lethal mutations in *Drosophila* at concentrations 220–230 times that of a normal cup of tea (Neethling et al., 1988). Regression analyses, determining a theoretical mutation frequency, failed to indicate any practical implications regarding the mutagenic effects of the tea at normal drinking concentrations. It was suggested that a minor component of rooibos, quercetin, could be responsible, as it is known to be mutagenic in different assay systems including the *Salmonella* test (Snijman et al., 2007). Quercetin, however, occurs widely in a variety of food sources and is readily detected in the blood of humans (Manach et al., 2004). Thus is seems unlikely that the mutagenic effect of rooibos would be relevant to tea drinkers when considering the quantities consumed.

Herb–drug interactions have become very important clinically. The effect on pharmacokinetics of a drug might vary when co-administered in the presence of a specific herb. This would imply that the concentrations of a drug in the tissue could be either increased or decreased leading to toxicity or decreased efficacy and failure of treatment, respectively. In order to promote the health benefits of the South African herbal teas, it must be determined whether any herb–drug interactions occur that could adversely affect drug metabolism. Two studies in rats investigated the effect of rooibos on the expression of CYPs. Treatment of rats with a rooibos infusion for a period of 3 days inhibited the expression of CYP2C11, known to metabolise a variety of drugs which increases their half life, possibly resulting in toxicity (Jang et al., 2004). Another study in rats also indicated that rooibos administered in their drinking water selectively increased the activity and expression of CYP3A in the intestine, which significantly increased the hydroxylation of the drug midazolam, a short acting benzodiazepine derivative (Matsuda et al., 2007). CYP3A is a key enzyme in the metabolism of a large number of therapeutic drugs.

This is of interest as it was shown that rooibos and certain honeybush flavonoids selectively induced CYP P450 isozymes, especially CYP3A4 that metabolise many drugs. Several drug classes including the calcium antagonists and the hydroxymethylglutaryl-coenzyme A reductase inhibitors are affected (review by Mertens-Talcott et al., 2006). Studies on hesperidin and its aglycone hesperetin, flavonoids of citrus and honeybush, were shown to alter the

effect of cancer chemotheurapeutic drugs. Hesperetin increased the transport of vincristine across the blood–brain barrier while hesperidin interacted with daunomycin (review by Garg et al., 2001). Other honeybush flavonoids, naringenin and naringin inhibited P-glycoprotein and organic anion-transporting polypeptides which are efflux and influx transporters, respectively, affecting drug bioavailibility (Mertens-Talcott et al., 2006).

Many species of Asteraceae contain toxic pyrrolizidine alkaloids, which prompted McGaw et al. (2007) to test for the presence of these compounds in *Athrixia phylicoides* and *Athrixia elata* aerial parts. The absence of these compounds, as well as caffeine, was confirmed using spectrophotometric and GC-MS analysis. In addition, the cytotoxicity of an infusion, decoction, cold water extract and cold ethanol extract of both species were tested against the Vero monkey kidney cell line and in the brine shrimp larval mortality assay (McGaw et al., 2007). Ethanol extracts were found to be cytotoxic in both assays with $LC_{50} < 400$ μg/mL, while all water extracts exhibited LC_{50} values > 1000 μg/mL.

Although bush tea has a long history of traditional use and has been traded informally for many years, it was only recently considered for formal commercialisation. However, the herbal tea industry is now less inclined to rely on traditional use as indication of safety and absence of toxicity. This prompted a sub-chronic toxicity study of bush tea. A water extract of *Athrixia phylicoides* (30, 90 and 180 mg/kg body mass per day) was tested for subchronic toxicity in a 90-day feeding study using Wistar rats (Chellan et al., 2007). No morbidity or mortality was observed. Food and water intake, as well as body mass and stool production, were unaffected. A mild diuretic effect was observed for the higher dosages. The biochemical parameters, alkaline phosphatase, creatinine and urea levels, were normal in the serum and no signs of toxicity were observed by histopathology of the liver, kidneys and gastro-intestinal tract.

9. Conclusions and future research

Enjoyment of rooibos and honeybush as beverages consumed for their flavour forms the basis of current product demand, however research during the past decade on health promoting properties to support a historical "healthy image", has greatly contributed to increased consumption. The focus of research has primarily been directed by consumer interest in antioxidants and their beneficial properties, which supports the "food as medicine" concept. Lower *in vitro* antioxidant activity for honeybush infusions in comparison to rooibos infusions has stimulated the search for therapeutic uses not linked to antioxidant activity. In this regard the research on the phyto-oestrogenic properties of honeybush showed promising results.

Although beneficial effects have been demonstrated for rooibos and honeybush in *in vitro* and *in vivo* models, human studies are either very limited in the case of rooibos or non-existing for honeybush. Most of these studies on the biological properties do not include information on the chemical composition of the infusions or extracts used. This information is critical for understanding the link between the biological effects and composition. Variation in efficacy of extracts underlines the need for caution when claims are made, as well as a need for chemical and/or biological markers to be used for standardisation.

Several aspects should receive attention to fully realise the potential of rooibos and honeybush as health-promoting beverages, or as sources of bioactive extracts or compounds. The bioactivity of their polyphenolic compounds, especially the major antioxidants, in target tissues is speculative at this stage. Investigation of the bioavailability, tissue distribution and biological activities of metabolites of the bioactive polyphenols is therefore required. As the presence of other polyphenols, sugars, etc. would influence their absorption and thus bioavailability, results from one type of plant extract cannot, without reservation, be extrapolated to another.

Possible herb–drug interactions, of great concern in the dietary supplement and nutraceutical industries, could affect drug efficacy and/or potency, due to either lower than expected plasma concentrations or toxic levels, and should also be investigated.

From an agricultural perspective, improved plant material through selection and breeding with regards to productive lifespan, composition (less variable and higher concentrations of bioactives) and bioactivity is required.

Biological properties summarised for rooibos and honeybush in this review focussed primarily on *in vitro* and animal studies. Final confirmation of these health-promoting properties requires validation in humans to ensure continued market interest.

Acknowledgements

The authors thank SA Rooibos Council for providing information of up-to-date rooibos production areas, collected during their Sustainable Rooibos Initiative, Marlise Joubert of ARC Infruitec-Nietvoorbij for information on honeybush distribution areas collected during 2007 for a Geographical Information System survey, Ronelda Jacobs of Resource Utilization, Department of Agriculture Western Cape, Elsenburg for preparing the composite map and Ina Olivier and Rita Sauders of ARC Infruitec-Nietvoorbij for the final artwork.

References

Adlercreutz, H., Mazur, W., 1997. Phyto-oestrogens and Western diseases. Annals of Medicine 29, 95–120.
Akaike, T., Ijiro, S., Sato, K., Katsuki, T., Maeda, H., 1995. Determination of peroxyl radical-scavenging activity in food by using batericidal action of alkyl peroxyl radical. Journal of Agricultural and Food Chemistry 43, 1864–1870.
Almajano, M.P., Carbó, R., Jiménez, J.A.L., Gordon, M.H., 2008. Antioxidant and antimicrobial activities of tea infusions. Food Chemistry 108, 55–63.
Amin, A., Buratovich, M., 2007. The anti-cancer charm of flavonoids: a cup of tea will do! Recent Patents on Anti-Cancer Drug Discovery 2, 1–9.
Anon., 1967. Rooibos Tea Control Board 12th Annual Report. Clanwilliam, South Africa.
Anon., 1991. Rooibos Tea Board Annual Report. Clanwilliam, South Africa.
Anon., 1994. Voorrade situasie. Die Rooibos No. 2, p. 4.
Anon., 2005a. Unique functional rooibos ingredient. South African Food Review 32, 21.
Anon., 2005b. Cognis offers rooibos tea extract, expands plant line. Foodnavigator Electronic Newsletter (December 1).
Anon., 2006. Is that rooibos in my cappuccino? South African Food Review 33, 24.
Anon., 2008. Athrixia. http://www.biodiversityexplorer.org/plants/asteraceae/athrixia.htm (downloaded on April 30, 2008).
Araya, H.T., 2005. Seed germination and vegetative propagation of bush tea (*Athrixia phylicoides*). M.Sc. Agric (Horticulture) Thesis. University of Pretoria, Pretoria, South Africa.
ARC, 2008. Website of Honeybush Research Programme. Agricultural Research Council, South Africa. http://www.arc.agric.za/home.asp?pid=4045 (downloaded on April 30, 2008).
Baranska, M., Schulz, H., Joubert, E., Manley, M., 2006. *In situ* flavonoid analysis by FT-Raman spectroscopy: Identification, distribution, and quantification of aspalathin in green rooibos (*Aspalathus linearis*). Analytical Chemistry 78, 7716–7721.
Blommaert, K.L.J., Steenkamp, J., 1978. Tannien- en moontlike kafeïeninhoud van rooibostee, *Aspalathus* (Subgen. Nortiera) *linearis* (Burm. Fil) R. Dahlgr. Agroplantae 10, 49.
Bohlmann, F., Wallmeyer, M., Jakupovic, J., 1982. A new seco-labdane derivative from *Athrixia elata*. Phytochemistry 21, 1806–1807.
Bohlmann, F., Zdero, C., 1977. Neue norkauren- und thymol-derivate aus *Athrixia*-arten. Phytochemistry 16, 1773–1776.
Bond, P., Goldblatt, P., 1984. Plants of the Cape Flora. A Descriptive Catalogue. CTP Book Printers, Cape Town, South Africa, pp. 285–286.
Botha, M., 2005. Use of near infrared spectroscopy (NIRS) and spectrophotometric methods in quality control of green rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia genistoides*). M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Bowie, J., 1830. Sketches of the botany of South Africa. South African Quarterly Journal I, 27–36.
Bramati, L., Aquilano, F., Pietta, P., 2003. Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC–UV and determination of the total antioxidant activity. Journal of Agricultural and Food Chemistry 51, 7472–7474.
Bramati, L., Minoggio, M., Gardana, C., Simonetti, P., Mauri, P., Pietta, P., 2002. Quantitative characterization of flavonoid compounds in rooibos tea (*Aspalathus linearis*) by LC–UV-DAD. Journal of Agricultural and Food Chemistry 50, 5513–5519.
Breet, P., Kruger, H.S., Jerling, J.C., Oosthuizen, W., 2005. Actions of black tea and rooibos on the iron status of primary school children. Nutrition Research 25, 983–994.
Cassidy, A., Hanley, B., Lamuela-Raventos, R.M., 2000. Isoflavones, lignans and stilbenes—origins, metabolism and potential importance to human health. Journal of the Science of Food and Agriculture 80, 1044–1062.
Chellan, N., De Beer, D., Muller, C., Joubert, E., Louw, J., 2007. A toxicological assessment of *Athrixia phylicoides* aqueous extract following sub-chronic ingestion in a rat model. In: Abstracts of the Pharmacology and Toxicology Congress, Marikana, South Africa, October 2007, p. 42.
Cheney, R.H., Scholtz, E., 1963. Rooibos tea, a South African contribution to world beverages. Economic Botany 17, 186–194.
Coetzee, G., Marx, I.J., Pengilly, M., Bushula, V.S., Joubert, E., Bloom, M., 2008. Effect of rooibos and honeybush tea extracts against *Botrytis cinerea*. South African Journal of Enology and Viticulture 29, 33–38.
Coetzee, W.H.K., Van der Merwe H.C., Burger, I.J., 1953. Die samestelling van rooibostee. Boerdery in Suid-Afrika 28, 95–99.
Cornwell, T., Cohick, W., Raskin, I., 2004. Dietary phytoestrogens and health. Phytochemistry 65, 995–1016.
Dahlgren, R., 1964. The correct name of the "rooibos" tea plant. Botaniska Notiser 117, 188–196.
Dahlgren, R., 1968. Revision of the genus *Aspalathus*. II. The species with ericoid and pinoid leaflets. 7. Subgenus Nortieria. With remarks on rooibos tea cultivation. Botaniska Notiser 121, 165–208.
Dahlgren, R., 1988. Crotalarieae (*Aspalathus*). Flora of Southern Africa 16, 84–90.
De Beer, S.W., Joubert, E., 2002. Preparation of Tea-like Beverages. SA Patent No. 2002/2802.
De Beer, D., Joubert, E., 2007. Antioxidant potential and phenolic composition of bush tea (*Athrixia phylicoides*) extracts. In: Abstracts of Indigenous Plant Use Forum, Johannesburg, South Africa, July 2007, p. 30.
De Lima Toccafondo Vieira, M., Ferreira Duarte, R., Moreira Campos, L.M., de Aguiar Nunan, E., 2008. Comparison of the estrogenic potencies of standardized soy extracts by immature rat uterotrophic bioassay. Phytomedicine 15, 31–37.
De Nysschen, A.M., Van Wyk, B.-E., Van Heerden, F.R., Schutte, A.L., 1996. The major phenolic compounds in the leaves of *Cyclopia* species (Honeybush tea). Biochemical and Systematic Ecology 24, 243–246.
Dixon, R.A., 2004. Phytoestrogens. Annual Review of Plant Biology 55, 225–261.
Dos, A., Ayhan, Z., Sumnu, G., 2005. Effects of different factors on sensory attributes, overall acceptance and preference of rooibos (*Aspalathus linearis*) tea. Journal of Sensory Studies 20, 228–242.
Du Toit, J., Joubert, E., 1998a. Effect of drying conditions on the quality of honeybush tea (*Cyclopia*). Journal of Food Processing Preservation 22, 493–507.
Du Toit, J., Joubert, E., 1998b. The effect of pretreatment on the fermentation of honeybush tea (*Cyclopia maculata*). Journal of the Science of Food and Agriculture 76, 537–545.
Du Toit, J., Joubert, E., 1999. Optimization of the fermentation parameters of honeybush tea (*Cyclopia*). Journal of Food Quality 22, 241–256.
Du Toit, J., Joubert, E., Britz, T.J., 1998. Honeybush tea—a rediscovered indigenous South African herbal tea. Journal of Sustainable Agriculture 12, 67–84.
Du Toit, J., Joubert, E., Britz, T.J., 1999. Identification of microbial contaminants present during the curing of honeybush tea (*Cyclopia*). Journal of the Science of Food and Agriculture 79, 2040–2044.
Du Toit, R., Volsteedt, Y., Apostolides, Z., 2001. Comparsion of the antioxidant content of fruits, vegetables and teas as vitamin C equivalents. Toxicology 166, 63–69.
Edenharder, R., Sager, J.W., Glatt, H., Muckel, E., Platt, K.L., 2002. Protection by breverages, fruits, vegetables, herbs, and flavonoids against genotoxicty of 2-acetylaminofluorene and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyrydine (PhIP) in metabolically competent V79 cells. Mutation Research 521, 57–72.
Ferreira, D., Kamara, B.I., Brandt, E.V., Joubert, E., 1998. Phenolic compounds from *Cyclopia intermedia* (honeybush tea). 1. Journal of Agricultural and Food Chemistry 46, 3406–4310.
Ferreira, D., Marais, C., Steenkamp, J.A., Joubert, E., 1995. Rooibos tea as a likely health food supplement. In: Proceedings of the Recent Development of Technologies on Fundamental Foods for Health, Korean Society of Food Science and Technology, Seoul, Korea, pp. 73–88.
Fouche, G., Khorombi, E., Kolesnikova, N., Maharaj, V.J., Nthambeleni, R., Van der Merwe, M., 2006. Investigation of South African plants for anticancer properties. Pharmacology online 3, 494–500.
Fox, F.W., Young, M.E.N., 1982. Food from the Veld. Edible Wild Plants of Southern Africa. Delta Books (Pty) Ltd., Johannesburg, South Africa, pp. 119–120.
Garg, A., Garg, S., Zaneveld, L.J.D., Singla, A.K., 2001. Chemistry and pharmacology of the citrus bioflavonoid hesperidin. Phytotheraphy Research 15, 655–669.
Gilani, A.H., Khan, A., Ghayur, M.N., Ali, S.F., Herzig, J.W., 2006. Antispasmodic effect of rooibos tea (*Aspalathus linearis*) is mediated through K^+ channel activation. Basic Clinical Pharmacology and Toxicology 99, 365–373.
Greenish, H.G., 1881. Cape tea. The Pharmaceutical Journal and Transactions 11, 549–551.
Grüner, S., Otto, F., Weinreich, B., 2003. Honeybush (*Cyclopia sessiliflora*)—a source of polyphenols from South Africa. In: Supplement to Abstract Book—1st International Conference on Polyphenols and Health, 18–21 November 2003, Vichy, France, p. 14.
Guardia, T., Rotelli, A.E., Juarez, A.O., Pelzer, L.E., 2003. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Pharmacology 56, 683–687.
Habu, T., Flath, R.A., Mon, T.R., Morton, J.F., 1985. Volatile components of rooibos tea (*Aspalathus linearis*). Journal of Agricultural and Food Chemistry 33, 249–254.
Hesseling, P.B., Joubert, J.R., 1982. The effect of rooibos tea on the type I allergic reaction. South African Medical Journal 62, 1037–1038.
Hesseling, P.B., Klopper, J.F., Van Heerden, P.D.R., 1979. Die effek van rooibostee op ysterabsorpsie. South African Medical Journal 55, 631–632.
Hillis, W.E., Inoue, T., 1967. The polyphenols of *Nothofagus* species. II. The heartwood of *Nothofagus fusca*. Phytochemistry 6, 59–67.
Hitomi, E., Nishikiori, A., Matsumoto, A., Moriguchi, S., Kojo, S., Tokumaro, S., Nakano, M., 2004. Effect of rooibos tea (*Aspalathus linearis*) extract on lipid peroxidation in vitamin E defiecient rats. ITE Letters on Batteries, New Technology and Medicine 5, 64–72.
Hitomi, E., Tamura, S., Tsurumoto, Y., Tsuda, T., Nakano, M., 1999. Antioxidant activity of rooibos tea (*Aspalathus linearis*). Journal of the Japanese Society for Food Science and Technology 46, 779–785 (CAB Abstract).
Hofmeyer, J., Phillips, E.P., 1922. The genus *Cyclopia*. Vent. Bothalia 1, 105–109.
Holmes, D.J., Austad, S.N., 1995. Birds as animal models for comparative biology of ageing: a prospectus. Journals of Gerontology, Series A: Biological Sciences and Medical Sciences 50A, B59–B66.
Huang, M., Du Plessis, J., Du Preez, J., Hamman, J., Viloen, A., 2008. Transport of aspalathin, a rooibos tea flavonoid, across the skin and intestinal epithelium. Phytotherapy Research 22, 669–704.
Hubbe, M.H., 2000. Evaluation of the antioxidant and free radical scavenging activities of honeybush tea (*Cyclopia*). M.Sc. (Biochemistry) Thesis. Stellenbosch University, Stellenbosch, South Africa.
Hubbe, M.H., Joubert, E., 2000. *In vitro* superoxide anion radical scavenging ability of honeybush tea (*Cyclopia*). In: Johnson, I.T., Fenwick, G.R. (Eds.), Dietary Anticarcinogens and Antimutagens. Chemical and Biological Aspects (Proceedings of the Food and Cancer Prevention III Meeting, 5–8 September 1999). Royal Chemistry Society, UK, pp. 242–244.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg, South Africa.
Ichiyama, K., Tai, A., Yamamoto, I., 2007. Augmentation of antigen-specific antibody production and IL-120 generation with a fraction from rooibos (*Aspalathus linearis*) tea. Bioscience Biotechnology and Biochemistry 71, 589–602.
Inanami, O., Asanuma, T., Inukai, N., Jin, T., Shimokawa, S., Kasai, N., Nakano, M., Sato, F., Kuwabara, M., 1995. The suppression of age-related accumulation of lipid peroxides in rat brain by administration of rooibos tea (*Aspalathus linearis*). Neuroscience Letters 196, 85–88.
Ito, A., Shinohara, K., Kator, K., 1991. Protective action of rooibos tea (*Aspalathus linearis*) extracts against inactivation of L5178Y cells by H_2O_2. In: Proceedings of the International Symposium on Tea Science, The Organizing Committee of ISTS, Shizuoka, Japan, pp. 381–384.
Ivanova, D., Gerova, D., Chervenko, T., Yankova, T., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology 96, 145–150.
Jaganyi, D., Wheeler, P.J., 2003. Rooibos tea: equilibrium and extraction kinetics of aspalathin. Food Chemistry 83, 121–126.
Jang, E.-H., Park, Y.-C., Chung, W.-G., 2004. Effects of dietary supplements on the induction and inhibition of cytochrome P450s protein expression in rats. Food Chemical Toxicology 42, 1749–1756.
Joubert, E., 1984. Studies in verband met verkitsing van rooibostee (*Aspalathus linearis*). M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.
Joubert, E., 1988a. Effect of agglomeration on the properties of spary-dried rooibos tea. International Journal of Food Science and Technology 23, 203–207.
Joubert, E., 1988b. Effect of batch extraction conditions on yield of soluble solids from rooibos tea. International Journal of Food Science and Technology 23, 43–47.
Joubert, E., 1990a. Chemical and sensory analyses of spray- and freeze-dried extracts of rooibos tea (*Aspalathus linearis*). International Journal of Food Science and Technology 25, 344–349.
Joubert, E., 1990b. Effect of batch extraction conditions on yield of polyphenols from rooibos tea (*Aspalathus linearis*). International Journal of Food Science and Technology 25, 339–343.
Joubert, E., 1990c. Effect of time on fixed-bed extraction of polyphenols from rooibos tea (*Aspalathus linearis*). Lebensmitteln-Wissenschaft und -Technologie 23, 181–183.
Joubert, E., 1994. Processing of rooibos tea under controlled conditions (*Aspalathus linearis*). Ph.D. (Food Science) Dissertation. Stellenbosch University, Stellenbosch, South Africa.
Joubert, E., 1995. Tristimulus colour measurement of rooibos tea extracts as an objective colour quality parameter. International Journal of Food Science and Technology 30, 783–792.

Joubert, E., 1996. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. Food Chemistry 55, 403–411.

Joubert, E., 1998. Effect of controlled conditions during deep bed fermentation and drying on rooibos tea (*Aspalathus linearis*) quality. Journal of Food Processing and Preservation 22, 405–417.

Joubert, E., De Villiers, O.T., 1997. Effect of fermentation and drying conditions on the quality of rooibos tea. International Journal of Food Science and Technology 32, 127–134.

Joubert, E., Hansmann, C.F., 1990. Effect of time on fixed-bed extraction of soluble solids from rooibos tea (*Aspalathus linearis*). Lebensmitteln-Wissenschaft und -Technologie 23, 178–180.

Joubert, E., Manley, M., Botha, M., 2006a. Use of NIRS for quantification of mangiferin and hesperidin contents of dried green honeybush (*Cyclopia genistoides*) plant material. Journal of Agricultural and Food Chemistry 54, 5279–5283.

Joubert, E., Manley, M., Botha, M., 2008a. Evaluation of spectrophotometric methods for screening of green rooibos (*Aspalathus linearis*) and green honeybush (*Cyclopia genistoides*) extracts for high levels of bio-active compounds. Phytochemical Analysis 19, 169–178.

Joubert, E., Müller, R., 1997. A small-scale rotary fermentation unit for rooibos tea. Inter national Journal of Food Science and Technology 32, 135–139.

Joubert, E., Otto, F., Grüner, S., Weinreich, B., 2003. Reversed-phase HPLC determination of mangiferin, isomangiferin and hesperidin in *Cyclopia* and the effect of harvesting date on the phenolic composition of *C. genistoides*. European Food Research and Technology 216, 270–273.

Joubert, E., Richards, E.S., Van der Merwe, J.D., De Beer, D., Manley, M., Gelderblom, W.C.A., 2008b. Effect of species variation and processing on phenolic composition and *in vitro* antioxidant activity of aqueous extracts of *Cyclopia* spp. (honeybush tea). Journal of Agricultural and Food Chemistry 56, 954–963.

Joubert, E., Schulz, H., 2006. Production and quality aspects of rooibos tea and quality products. A review. Journal of Applied Botany and Food Quality 80, 138–144.

Joubert, E., Van der Merwe, J.D., Gelderblom, W.C.A., Manley, M., 2006b. Implication of CYP450 stabilization in the evaluation of in vitro antimutagenicity of the herbal teas, *Cyclopia* spp. (honeybush) and *Aspalathus linearis* (rooibos) and selected polyphenols. In: Polyphenols Communications 2006: Abstracts of XXIIird International Conference on Polyphenols, 22–25 August 2006, Winnipeg, Manitoba, Canada, pp. 505–506.

Joubert, E., Winterton, P., Britz, T.J., Ferreira, D., 2004. Superoxide anion radical and α,α-diphenyl-β-picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. Food Research International 37, 133–138.

Joubert, E., Winterton, P., Britz, T.J., Gelderblom, W.C.A., 2005. Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (*Aspalathus linearis*). Journal of Agricultural and Food Chemistry 53, 10260–10267.

Juráni, M., Lamošová, D., Máčajová, M., Kostál, L., Joubert, E., Greksák, M., 2008. Effect of rooibos tea (*Aspalathus linearis*) on Japanese quail growth, egg production and plasma metabolites. British Poultry Science 49, 55–64.

Kamara, B.I., Brand, D.J., Brandt, E.V., Joubert, E., 2004. Phenolic metabolites from honeybush tea (*Cyclopia subternata*). Journal of Agricultural and Food Chemistry 52, 5391–5395.

Kamara, B.I., Brandt, E.V., Ferreira, D., Joubert, E., 2003. Polyphenols from honeybush tea (*Cyclopia intermedia*). Journal of Agricultural and Food Chemistry 51, 3874–3879.

Kawakami, M., Kobayashi, A., Kator, K., 1993. Volatile constituents of rooibos tea (*Aspalathus linearis*) as effected by extraction process. Journal of Agricultural and Food Chemistry 41, 633–636.

Kazuno, S., Yanagida, M., Shindo, N., Murayama, K., 2005. Mass spectrometric identification and quantification of glycosyl flavonoids, including dihydrochalcones with neutral loss scan mode. Analytical Biochemistry 347, 182–192.

Kelly, K.M., Van Staden, J., 1985. Effect of acid scarification on seed coat structure, germination and seedling vigour of *Aspalathus linearis*. Journal of Plant Physiology 121, 37–45.

Khan, A., Gilani, A.H., 2006. Selective broncholdilatory effect of rooibos tea (*Aspalathus linearis*) and its flavonoid, chrysoeriol. European Journal of Nutrition 45, 463–469.

Kies, P., 1951. Revision of the genus *Cyclopia* and notes on some other sources of bush tea. Bothalia 6, 161–176.

Kinae, N., Shimoi, K., Masumori, S., Harasawa, M., Furugori, M., 1994. Suppression of the formation of advanced glycosylation products by tea extracts. In: Ho, C.-T., Osawa, T., Huang, M.-T., Rosen, R.T. (Eds.), Food Phytochemicals for Cancer Prevention II, ACS Symposium Series, vol. 547. American Chemical Society, Washington, DC, USA, pp. 68–75.

Kobert, R., 1906. Lehrbuch der Intoxikationen, 2nd ed., ii (2). pp. 748, 1029 (as cited by Watt and Breyer-Brandwijk, 1932).

Koeppen, B.H., Roux, D.G., 1965a. Aspalathin: a novel *C*-glycosylflavonoid from *Aspalathus linearis*. Tetrahedron Letters 39, 3497–3503.

Koeppen, B.H., Roux, D.G., 1965b. *C*-Glycosylflavonoids. The chemistry of orientin and *iso*-orientin. Biochemistry Journal 97, 444–448.

Koeppen, B.H., Roux, D.G., 1966. *C*-Glycosylflavonoids. The chemistry of aspalathin. Biochemistry Journal 99, 604–609.

Koeppen, B.H., Smit, C.J.B., Roux, D.G., 1962. The flavone *C*-glycosides and the flavanol *O*-glycosides of *Aspalathus acuminatus* (Rooibos tea). Biochemistry Journal 83, 507–511.

Komatsu, K., Kator, K., Mitsuda, Y., Mine, M., Okumura, Y., 1994. Inhibitory effects of rooibos, *Aspalathus linearis*, on X-ray-induced C3H10T1/2 cell transformation. Cancer Letters 77, 33–38.

Krafczyk, N., Glomb, M.A., 2008. Characterization of phenolic compounds in rooibos tea. Journal of Agricultural and Food Chemistry 56, 3368–3376.

Kucharská, J., Uličná, O., Gvozdjáková, A., Sumbalová, Z., Vančová, O., Božek, P., Nakano, M., Greksák, M., 2004. Regeneration of coenzyme Q_9 redox state and inhibition of oxidative stress by rooibos tea (*Aspalathus linearis*) administration in carbon tretrachloride liver damage. Physiology Research 53, 515–521.

Kunishiro, K., Tai, A., Yamamoto, I., 2001. Effects of rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. Bioscience Biotechnology and Biochemistry 65, 2137–2145.

Kurzer, M.S., Xu, X., 1997. Dietary phytoestrogens. Annual Review of Nutrition 17, 353–381.

Lamošová, D., Juráni, M., Greksák, M., Nakano, M., Vaneková, M., 1997. Effect of rooibos tea (*Aspalathus linearis*) on chick skeletal cell growth in culture. Comparative Biochemistry and Physiology 116C, 39–45.

Laughton, M.J., Halliwell, B., Evans, P.J., Hoult, J.R.S., 1989. Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. Biochemical Pharmacology 38, 2859–2865.

Lee, E.-J., Jang, H.-D., 2004. Antioxidant activity and protective effect on DNA strand scission of rooibos tea (*Aspalathus linearis*). BioFactors 21, 285–292.

Leiro, J.M., Álvarez, E., Arranz, J.A., Siso, I.G., Orallo, F., 2003. in vitro effects of mangiferin on superoxide concentrations and expression of the inducible nitric oxide synthase, tumour necrosis factor-α and transforming growth factor-β genes. Biochemical Pharmacology 65, 1361–1371.

Leistner, O.A., 2000. Seed Plants of Southern Africa: Families and Genera, Strelitzia, vol. 10. National Botanical Institute, Pretoria, South Africa, p. 125.

Le Roux, M., Cronje, J.C., Joubert, E., Burger, B.V., 2008. Chemical characterization of the consitutents of the aroma of honeybush, *Cyclopia genistoides*. South African Journal of Botany 74, 139–143.

Lindsey, K.L., Motsei, M.L., Jäger, A.K., 2002. Screening of South African food plants for antioxidant activity. Journal of Food Science 67, 2129–2131.

Magee, P.J., Rowland, I.R., 2004. Phyto-oestrogens, their mechanism of action: current evidence for a role in breast and prostate cancer. British Journal of Nutrition 91, 513–531.

Maicu, M.C., 2008. Optimistion of retention of mangiferin in *Cyclopia subternata* during preparation for drying and storage of green honeybush and development of NIR spectroscopy calibration models for rapid quantification of mangiferin and xanthones contents. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Mak, P., Leungy, Y.-K., Tangy, W.-T., Harwood, C., Hoy, S.-M., 2006. Apigenin suppresses cancer cell growth through ERβ. Neoplasia 8, 896–904.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., Jiménez, L., 2004. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition 79, 727–747.

Manley, M., Joubert, E., Botha, M., 2006. Quantification of the major phenolic compounds, soluble solids content and total antioxidant activity of green rooibos (*Aspalathus linearis*) by means of near infrared spectroscopy. Journal of Near Infrared Spectroscopy 14, 213–222.

Marais, C., Janse van Rensburg, W., Ferreira, D., Steenkamp, J.A., 2000. (*S*)- and (*R*)-Eriodictyol-6-*C*-β-D-glucopyranoside, novel keys to the fermentation of rooibos (*Aspalathus linearis*). Phytochemistry 55, 43–49.

Marais, S.S., Marais, C., Steenkamp, J.A., Malan, E., Ferreira, D., 1998. Progress in the investigation of rooibos tea extractives. In: Gross, G.G., Hemingway, R.W., Yoshida, T. (Eds.), Abstracts of the 3rd Tannin Conference. Bend, Oregon, USA, pp. 129–130.

Marais, C., Steenkamp, J.A., Ferreira, D., 1996. The occurrence of phylpyruvic acid in woody plants: Biosynthetic significance. Tetrahedron Letters 37, 5763–5764.

Markaverich, B.M., Gregory, R.R., 1993. Preliminary assessment of luteolin as an affinity ligand for type II estrogen-binding sites in rat uterine nuclear extracts. Steroids 58, 268–274.

Marloth, R., 1909. The chemistry of some vegetable products of South Africa. Agricultural Journal 35, 634–638.

Marloth, R., 1913. The Chemistry of South African Plants and Plant Products. Cape Chemical Society, Cape Town, p. 9.

Marloth, R., 1925. The Flora of South Africa with Synoptical Tables of the Genera of the Higher Plants. Darter Bros & Co., Cape Town, South Africa, pp. 69–72.

Marnewick, J.L., 2004. Chemopreventive properties of two South African herbal teas, rooibos tea (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) utilizing different short-term *in vivo* and *in vitro* carcinogenesis models. Ph.D. (Biochemistry) Dissertation. University of Stellenbosch, Stellenbosch, South Africa.

Marnewick, J.L., Batenburg, W., Swart, P., Joubert, E., Swanevelder, S., Gelderblom, W.C.A., 2004. *Ex vivo* modulation of chemical-induced mutagenesis by subcellular liver fractions of rats treated with rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) as well as green and black (*Camellia sinensis*) teas. Mutation Research 558, 145–154.

Marnewick, J.L., Joubert, E., Joseph, S., Swanevelder, S., Swart, P., Gelderblom, W.C.A., 2005. Inhibition of tumour promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), unique South African herbal teas. Cancer Letters 224, 193–202.

Marnewick, J.L., Joubert, E., Swart, P., Van der Westhuizen, F., Gelderblom, W.C.A., 2003. Modulation of hepatic drug metabolizing enzymes and oxidative status by green and black (*Camellia sinensis*), rooibos (*Aspalathus linearis*) and honeybush

(*Cyclopia intermedia*) teas in rats. Journal of Agricultural and Food Chemistry 51, 8113–8119.
Marnewick, J.L., Gelderblom, W.C.A., Joubert, E., 2000. An investigation on the antimutagenic properties of South African herbal teas. Mutation Research 471, 157–166.
Mashimbye, M.J., Mudau, F.N., Soundy, P., Van Ree, T., 2006. A new flavonol from *Athrixia phylicoides* (bush tea). South African Journal of Chemistry 59, 1–2.
Matsuda, K., Nishimura, Y., Kurata, N., Iwase, M., Yasuhara, H., 2007. Effects of continuous ingestion of herbal teas on intestinal CYP3A in the rat. Journal of Pharmacological Science 103, 214–221.
McGaw, L.J., Steenkamp, V., Eloff, J.N., 2007. Evaluation of *Athrixia* bush tea for cytotoxicity, antioxidant activity, caffeine content and presence of pyrrolizidine alkaloids. Journal of Ethnopharmacology 110, 16–22.
Mertens-Talcott, S.U., Zadezensky, I., De Castro, W.V., Derendorf, H., Butterweck, V., 2006. Grapefruit-drug interactions: can interactions with drugs be avoided? Journal of Clinical Pharmacology 46, 1390–1416.
Mfenyana, C., 2008. Selective extraction of *Cyclopia* for enhanced *in vitro* phytoestrogenicity. M.Sc. (Biochemistry) Thesis. Stellenbosch University, Stellenbosch, South Africa.
Mokgalaka, N.S., McCrindel, R.I., Botha, B.M., 2004. Multielement analysis of tea leaves by inductively coupled plasma emission spectroscopy using slurry nebulisation. Journal of Analytical Atomic Spectroscopy 19, 1375–1378.
Morton, J.F., 1983. Rooibos tea, *Aspalathus linearis*, a caffeineless, low-tannin beverage. Economic Botany 37, 164–173.
Mudau, F.N., Soundy, P., Du Toit, E.S., 2005. Plant growth and development of bush tea as affected by nitrogen, phosphorus, and potassium nutrition. HortScience 40, 1898–1901.
Mudau, F.N., Soundy, P., Du Toit, E.S., Olivier, J., 2006. Variation in polyphenolic content of *Athrixia phylicoides* (L.) (bush tea) leaves with season and nitrogen application. South African Journal of Botany 72, 398–402.
Na, H.-K., Mossando, K.S., Lee, J.-Y., Surh, Y.-J., 2004. Inhibition of phorbol ester-induced COX-2 expression by some edible African plants. Biofactors 21, 149–153.
Nakano, M., Itoh, Y., Mizuno, T., Nakashima, H., 1997b. Polysaccharide from *Aspalathus linearis* with strong anti-HIV activity. Bioscience Biotechnology and Biochemistry 61, 267–271.
Nakano, M., Nakashima, H., Itoh, Y., 1997a. Anti-human immunodeficiency virus activity of oligosaccharides from rooibos tea (*Aspalathus linearis*) extracts in vitro. Leukemia 11 (supplement 3), 128–130.
Neethling, H.S., Theron, H.E., Geertsen, J.M.P., 1988. Die mutagenisiteit van rooibostee. South African Journal of Science 84, 278–279.
Nikolova, V., Petrova, S., Petkova, V., Pavlova, A., Georgieva, T., 2007. Antioxidant effects of rooibos tea on workers occupationally exposed to lead. Toxicology Letters 172 (S), 120–121.
Núñez-Sellés, A.J., 2005. Antioxidant therapy: myth or reality? Journal of the Brazilian Chemical Society 16, 699–710.
Oh, S.-M., Chung, K.-H., 2004. Estrogenic activities of *Ginkgo biloba* extracts. Life Sciences 74, 1325–1335.
Otto, F., Grüner, S., Weinreich, B., 2003a. Extract from "green" rooibos—a new raw material for the cosmetic industry. Euro Cosmetics, 20–23.
Otto, F., Grüner, S., Weinreich, B., 2003b. Rooibos (*Aspalathus linearis*)—development of an herbal extract with high antioxidative activity. In: Supplement to Abstract book—1st International Conference on Polyphenols and Health, 18–21 November 2003, Vichy, France, p. 13.
Pengilly, M., Joubert, E., Van Zyl, W.H., Botha, A., Bloom, M., 2008. Enhancement of rooibos (*Aspalathus linearis*) aqueous extract and antioxidant yield with fungal enzymes. Journal of Agricultural and Food Chemistry 56, 4047–4053.
Persson, I.A-L., Josefsson, M., Persson, K., Andersson, R.G.G., 2006. Tea flavanols inhibit antiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells. Journal of Pharmacy and Pharmacology 58, 1139–1144.
Piccinelli, A.L., De Simone, F., Passi, S., Rastrelli, L., 2004. Phenolic constituents and antioxidant activity of *Wendita calysina* leaves (Burrito), a folk Paraguyan tea. Journal of Agricultural and Food Chemistry 52, 5863–5868.
Rabe, C., Steenkamp, J.A., Joubert, E., Burger, J.F.W., Ferreira, D., 1994. Phenolic metabolites from rooibos tea (*Aspalathus linearis*). Phytochemistry 35, 1559–1565.
Rampedi, I., Olivier, J., 2005. The use and potential commercial development of *Athrixia phylicoides*. Acta Academica 37, 165–183.
Reynecke, J., Coetzee, W.H.K., Bester, J.J.A., 1949. Rooibos tea. A preliminary report on the composition. Farming in South Africa 24, 397–398, 409, 412.
Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20, 933–956.
Richfield, D., 2008. An investigation into the biological activity of rooibos (*Aspalathus linearis*) extracts. M.Sc. (Biochemistry) Thesis. Stellenbosch University, Stellenbosch, South Africa.
Rood, B., 1994. Uit die Veldapteek. Tafelberg-Uitgewers Bpk, Cape Town, South Africa, p. 51.
Rotelli, A.E., Guardia, T., Juarez, A.O., De la Rocha, N.E., Pelzer, L.E., 2003. Comparative study of flavonoids in experimental models of inflammation. Pharmacology Research 48, 601–606.
Rukachaisirikul, V., Naklue, W., Phongpaichit, S., Towatana, N.W., Maneenoon, K., 2006. Phloroglucinols, depsidones and xanthones from twigs of *Garcinia parvifolia*. Tetrahedron 62, 8578–8585.
Sasaki, Y.F., Yamada, H., Shimoi, K., Kator, K., Kinae, N., 1993. The clastogen-suppressing effects of green tea, Po-lei tea and rooibos tea in CHO cells and mice. Mutation Research 286, 221–232.
Sauter, W., 2004. Vergleich antioxidativer Eigenschaften von Extrakten aus *Camellia sinensis*, *Rosmarinus officinalis*, *Cyclopia genistoides*, *Cyclopia sessiliflora* und *Aspalathus linearis*. Ph.D. Dissertation. Technischen Universität München, Weihenstephan, Germany.
Scheepers, S., 2001. Anti-microbial activity of rooibos tea (*Aspalathus linearis*) on food spoilage organisms and potential pathogens. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.
Schulz, H., Joubert, E., Schütze, W., 2003. Quantification of quality parameters for reliable evaluation of green rooibos (*Aspalathus linearis*). European Food Research and Technology 216, 539–543.
Schutte, A.L., 1995. A taxonomic study of the tribes *Podalyrieae* and *Liparieae* (*Fabaceae*). Ph.D. Dissertation. Rand Afrikaans University, Johannesburg, South Africa.
Schutte, A.L., Vlok, J.H.J., Van Wyk, B.-E., 1995. Fire-survival strategy—a character of taxonomic, ecological and evolutionary importance in fynbos legumes. Plant Systematics and Ecology 195, 243–259.
Shimamura, N., Miyase, T., Umehara, K., Warashina, T., Fujii, S., 2006. Phytoestrogens from *Aspalathus linearis*. Biology and Pharmacology Bulletin 29, 1271–1274.
Shimoi, K., Hokabe, Y., Sasaki, Y.F., Yamada, H., Kator, K., Kinae, N., 1994. Inhibitory effect of rooibos tea on the induction of chromosome aberrations in vivo and in vitro. In: Ho, C.-T., Osawa, T., Huang, M.-T., Rosen, R.T. (Eds.), Food Phytochemicals for Cancer Prevention II, ACS Symposium Series, vol. 547. American Chemical Society, Washington, DC, USA, pp. 106–113.
Shimoi, K., Masuda, S., Shen, B., Furugori, M., Kinae, N., 1996. Radio protective effects of antioxidative plant flavonoids in mice. Mutation Research 350, 153–161.
Shindo, Y., Kato, K., 1991. Effect of rooibos tea on some dermatological diseases. In: Proceedings of the International Symposium on Tea Science, The Organizing Committee of ISTS, Shizuoka, Japan, pp. 385–389.
Silva, M.M., Santos, M.R., Caroço, G., Rocha, R., Justino, G., Mira, L., 2002. Structure–antioxidant activity relationships: a re-examination. Free Radical Research 36, 1219–1227.
Simon, M., Horovska, L., Greksak, M., Dusinsky, R., Nakano, M., 2000. Antihemolytic effect of rooibos tea (*Aspalathus linearis*) on red blood cells of Japanese quails. General Physiology and Biophysics 19, 365–371.
Sissing, 2008. Investigations into the cancer modulating properties of *Aspalathus linearis* (rooibos), *Cyclopia intermedia* (honeybush) and *Sutherlandia frutescens* (cancer bush) in oesophageal carcinogenesis. M.Sc. (Physiology) Thesis. University of the Western Cape, Bellville, South Africa.
Smith, C.A., Phillips, E.P., Van Hoeppen, E., 1966. Common Names of South African Plants. Botanical Survey Memoir No. 35. The Goverment Printer, Pretoria, South Africa, p. 247.
Snijman, P.W., 2007. Biological properties of selected flavonoids of rooibos. M.Sc. (Chemistry) Thesis. University of the Western Cape, Bellville, South Africa.
Snijman, P., Gelderblom, W., Joubert, E., Green, I., 2003. Biological activity of selected flavonoids of rooibos (*Aspalathus linearis*). In: Abstract Book—1st International Conference on Polyphenols and Health, 18–21 November 2003, Vichy, France, p. 358.
Snijman, P.W., Swanevelder, S., Joubert, E., Green, I.R., Gelderblom, W.C.A., 2007. The antimutagenic activity of the major flavonoids of rooibos (*Aspalathus linearis*)—some dose–response effects on mutagen activation–flavonoid interactions. Mutation Research 613, 111–123.
Snyckers, F.O., Salemi, G., 1974. Studies of South African medicinal plants. Part I. Quercetin as the major *in vitro* active compound of rooibos tea. Journal of the South African Chemical Institute 27, 5–7.
Standley, L., Winterton, P., Marnewick, J.L., Gelderblom, W.C.A., Joubert, E., Britz, T.J., 2001. Influence of processing stages on antimutagenic and antioxidant potentials of rooibos tea. Journal of Agricultural and Food Chemistry 49, 114–117.
Steenkamp, A., Fernandes, A.C., Van Rensburg, C.E.J., 2004. Antioxidant scavenging potential of South African export herbal teas. South African Journal of Botany 70, 660–663.
Terblanche, S.E., 1982. Report on Honeybush Tea. Department of Biochemistry, University of Port Elizabeth, Port Elizabeth, South Africa.
Tiedtke, J., Marks, O., 2002. Rooibos—the new "white tea" for hair and skin care. Euro Cosmetics (June 16–19).
Touyz, L.Z.G., Smit, A.A., 1982. Herbal tea infusions—their acidity, fluoride and calcium concentrations. Journal of the Dental Association of South Africa 37, 737–739.
Toyoda, M., Tanaka, K., Hoshino, K., Akiyama, H., Tanimura, A., Saito, Y., 1997. Profiles of potentially antiallergic flavonoids in 27 kinds of health tea and green tea infusions. Journal of Agricultural and Food Chemistry 45, 2561–2564.
Uličná, O., Greksák, M., Vančová, O., Zlatoš, L., Galbavý, Š., Božek, P., Nakano, M., 2003. Hepatoprotective effect of rooibos tea (*Aspalathus linearis*) on CCl_4-induced liver damage in rats. Physiology Research 52, 461–466.
Uličná, O., Vančová, O., Božek, P., Čársky, J., Šebeková, K., Boor, P., Nakano, M., Greksák, M., 2006. Rooibos tea (*Aspalathus linearis*) partially prevents oxidative stress in streptozotocin-induced diabetic rats. Physiology Research 55, 157–164.
Usui, T., 2006. Pharmaceutical prospects of phytoestrogens. Endocrine Journal 53, 7–20.
Van der Bank, M., Van Wyk, B.-E., Van der Bank, H., 1995. Biochemical genetic variation in four wild populations of *Aspalathus linearis* (rooibos tea). Biochemical Systematics and Ecology 23, 257–262.

Van der Merwe, J.D., 2004. A comparative study on protection of *Cyclopia* spp. (honeybush), *Aspalathus linearis* (rooibos) and *Camellia sinensis* teas against aflatoxin B1 induced mutagenesis in the *Salmonella* mutagenicity assay: possible mechanisms involved. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Van der Merwe, J.D., Joubert, E., Richards, E.S., Manley, M., Snijman, P.W., Marnewick, J.L., Gelderblom, W.C.A., 2006. A comparative study on the antimutagenic properties of aqueous extracts of *Aspalathus linearis* (rooibos), different *Cyclopia spp*. (honeybush) and *Camellia sinensis* teas. Mutation Research 611, 42–53.

Van Heerden, F.R., Van Wyk, B.-E., Viljoen, A.M., Steenkamp, P.A., 2003. Phenolic variation in wild populations of *Aspalathus linearis* (rooibos tea). Biochemical Systematics and Ecology 31, 885–895.

Van Wyk, B.-E., Gericke, M., 2000. People's Plants. A Guide to Useful Plants of Southern Africa. Briza Publications, Pretoria, South Africa, pp. 102–103.

Van Wyk, B.-E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa, p. 290.

Van Wyk, B.-E., Verdoorn, G.H., 1989. Alkaloids of the genera *Aspalathus*, *Rafnia* and *Wiborgia* (Fabaceae–Crotalarieae). South African Journal of Botany 55, 520–522.

Verhoog, N.J.D., Joubert, E., Louw, A., 2007a. Screening of four *Cyclopia* (honeybush) species for putative phyto-oestrogenic activity by oestrogen receptor binding assays. South African Journal of Science 103, 13–21.

Verhoog, N.J.D., Joubert, E., Louw, A., 2007b. Evaluation of the phytoestrogenic activity of *Cyclopia genistoides* (honeybush) methanol extracts and relevant polyphenols. Journal of Agricultural and Food Chemistry 55, 4371–4381.

Von Gadow, A., Joubert, E., Hansmann, C.F., 1997a. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. Food Chemistry 60, 73–77.

Von Gadow, A., Joubert, E., Hansmann, C.F., 1997b. Effect of extraction time and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. Journal of Agricultural and Food Chemistry 45, 1370–1374.

Von Gadow, A., Joubert, E., Hansmann, C.F., 1997c. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. Journal of Agricultural and Food Chemistry 45, 632–638.

Watt, J.M., Breyer-Brandwijk, M.G., 1932. The Medicinal and Poisonous Plants of Southern Africa. E & S Livingstone, Edinburgh, UK, p. 70.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. E & S Livingstone, Edinburgh, UK.

Whelan, A.M., Jurgens, T.M., Bowles, B.K., 2006. Natural health products in the prevention and treatment of osteoporosis: systemic review of randomized controlled trails. Complementary and Alternative Medicine 40, 836–848.

Winterton, P., 1999. Antioxidant activity of rooibos tea (*Aspalathus linearis*) in model lipid and radical generating systems. M.Sc. (Food Science) Thesis. Stellenbosch University, Stellenbosch, South Africa.

Yoo, K.M., Lee, C.H., Lee, H., Moon, B., Lee, C.Y., 2008. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry 106, 929–936.

Yoshikawa, T., Naito, Y., Oyamada, H., Ueda, S., Tanigawa, T., Takemura, T., Sugino, S., Kondo, M., 1990. Scavenging effects of *Aspalathus linearis* (rooibos tea) on active oxygen species. Experimental Medical Biology 264, 171–174.